

French at odds on new law

The law to regulate French universities has been signed — and opposing interests are already battling for influence over the decrees that will follow

EXCEPT for a little trouble with the French constitution, which should be resolved next month, France now has a new university law, the first major revision of university law since the student revolution of May 1968. But is the reform too little? Has a great opportunity been lost? The vociferous critics of the new law, notable among them Fields Medallist Laurent Schwartz of the Ecole Polytechnique, say loudly that it has been. Strangely enough, Schwartz's opponent at the ministry of education is another mathematician of similar intellectual temperament: Jean-Jacques Payan, ex-director-general of the principal French research council (CNRS) and ex-president of the University of Grenoble I, one of the foremost scientific universities of France. If Schwartz says the government has fumbled its chance, the man responsible is Payan. But Payan does not think he fumbled the ball but, instead, that he has made a pretty clever pass.

The differences between the two men, which epitomize the arguments over the future of the French university, are based less on differences of outlook than on differences of responsibility. Schwartz is a free spirit, outside the universities and with no power to exercise nor political pressure to which to adjust. Payan is in the hottest seat of all, in charge of the reform and acutely aware of what can and cannot be achieved. The new law is pragmatic, he admits, "but it proscribes little and permits much". Payan urges France to wait for the detailed, non-parliamentary "decree of application" which follows every law. Here may be some of the teeth, some of the support for research that Schwartz demands. "I have the same passion [as Schwartz]: the greatness of the French university", wrote Payan recently. So well do these conflicting passions set each other off than one must wonder whether Schwartz and Payan are in league, one creating the political climate that the other needs.

In reality, there are fundamental differences between the two men and the bodies of opinion they represent. Schwartz and those he speaks for wish to reintroduce selection in the first-year entry to university, the absence of which creates a "catastrophe" for both students and teachers. Payan and the law he represents avoid pre-entry selection and speak of post-entry "evaluation". Entry to the second and subsequent years will, in effect, be selective (as at present) but the word "evaluation" implies more: the universities should not evaluate the students according to some predetermined scheme but according to their students' needs, says Payan. Thus by implication in the new laws, the universities are to turn their resources towards their students, rather than the other way about. This is a revolution: it implies that the universities must become, at least in part, more like the existing technical institutes (IUT) or engineering schools (*grandes écoles*), for the students of the 1980s will certainly demand training that will lead to employment.

What of the dominance of French universities by trade unions and "corporatism"? Elections to various powerful committees of administration and promotion play a great — some say overwhelming — role in the post-1968 French university, and often these elections have been dominated by factional politics, with one union or body of staff against another. Schwartz says the new law worsens the situation. Payan, with some force, argues the reverse and, since he is no syndicalist, the benefit of the doubt must rest with him. According to Payan, the fact that under the new law elections will be by proportional representation, with

freedom for non-ascribed candidates, rather than the earlier winner-takes-all system, means that corporatism will be much diminished.

And what of research? Is it ignored by the law, as Schwartz says? Wait for the decree that will define the duties of university lecturers and professors, says Payan. Meanwhile, the text of the law introduces a sabbatical year — a welcome innovation for French scientists. It also establishes periodic evaluation of staff to keep them up to scratch, new recruitment procedures that "will leave little room for the establishment of local cliques" and imposes an obligation of mobility on professors. All this will improve the quality of French university research, says Payan. Moreover, the ministry is turning to a system of research support by multi-annual contracts, rather than the annual support related directly to student numbers presently in force. This should give the universities, traditionally dominated by central government, more academic independence.

As for the decree defining duties, that has been through many drafts and tense negotiations with the unions, and is now again being rewritten. The ministry says that it will take research "much more into account" than previous versions, meeting criticism from the presidents of the universities and others. Payan's early insistence on increased teaching duties and a longer working year will be somewhat softened and the duties are now being seen to be an overall requirement on a university, to be distributed according to some as yet undefined rules. The heavier teaching load will not be a direct requirement on individuals. The result of this, however, in conjunction with the law's insistence on free entry and on the evaluation of student need, may be to create a two-tier university structure in France. Some will teach, others will do research.

The pattern of research will also be transformed by the proposed change in the structure of higher degrees. The old *troisième cycle*, and the *thèse d'état*, respectively lesser versions of the PhD and DSc (the latter taking eight or nine years) are to be abandoned in favour of something approaching a PhD. This, most French scientists consider, will bring French science into line with the United States and Britain. But the new law has shrunk from out-and-out advocacy of meritocracy. As things are, would-be researchers can edge their way into laboratories on private means and eventually obtain a permanent post by waiting long enough, rather as in Italy. This system will continue even under the new law unless a comprehensive system of competitive grants is introduced. That step, still neglected, would do more than anything else to better the condition of science in France. □

Ivory tower in space?

NASA should be restrained from mounting a crash programme to build a US space station

THE National Aeronautics and Space Administration (NASA), set up in the heady days of the early 1960s, has always thrived on large engineering projects, undertaken on a crash basis with billions of dollars to throw about. Now that NASA has declared the shuttle operational (which, given recurring problems, including an explosion and fire after the shuttle's most recent



landing, is not the case), it is once again at a loose end — whence the plan to build a space station. Although nobody — not scientists, not the military, not even NASA itself — has been able to figure out just why the United States needs a space station, President Reagan is expected to approve the project when he releases his budget for fiscal year 1985 later this month.

Perhaps it was just a coincidence, but just before Christmas, Congress's Office of Technology Assessment released a report that seemed to answer NASA's prayers. The report, a sober description of the Soviet Salyut programme, has been seized upon to provide the ultimate justification for an American space station; the Soviets have one. Unfortunately, an American crash programme to build a space station will fail to correct the real problem in the US manned space programme that the report identifies, and in the process will also do considerable damage to American space science efforts — an area in which the United States really does lead the Soviets.

All the media hoopla over the red menace has ignored the more serious underlying failing of the US space programme compared with that of the Soviet Union: while the Soviets have plodded steadily forward towards a directed goal — a continuous manned presence in space, built up through mastery of automatic docking and long stays in space — the United States has gone in for space spectaculars, virtuoso efforts that reflect a dazzling technical ability and lead nowhere. The Apollo project (which, as a Tom Lehrer song has it, spent billions of dollars of taxpayers' money to put some clown on the moon) was not part of a larger plan.

The shuttle, also a crash programme that took the lion's share of NASA's resources, has suffered from a lack of a sense of direction. NASA, in a political decision to sell the shuttle to Congress, tied everything it could to the shuttle — if space science was needed, the shuttle would do space science; if it was military satellites, the shuttle would do military satellites. To make sure, NASA ditched its reliable expendable launch vehicles. The result was a disaster for space science, though not for the reasons usually given. The shuttle project itself probably did not steal funds away from science; what it did, however, was to delay by four years the Galileo mission to Jupiter (Galileo, of course, was designed to use the shuttle), and the funds needed to keep Galileo going for four years (it will finally be launched in 1986) eliminated the possibility of doing anything else in space science in the meanwhile. Also, the early promises of 28-day missions have given way to the reality that it does not make economic sense to park a billion-dollar launch vehicle in orbit for a month. The presence of men on a scientific platform can also be drawback, particularly for sensitive pointing apparatus. The shuttle was not needed for science; in many ways, it has hurt science.

The space station seems to be following the same path. And it will be a tragedy if science is once again constrained to suit NASA's political expediencies. If the space station goes ahead as another NASA crash programme, there will be another two decades of original research on why astronauts vomit and whether you can make the perfect ball-bearing in space.

If the Administration is truly concerned about staying ahead of the Soviet Union, it would do well to concentrate on the existing strengths in the US programme. The series of inexpensive near-Earth space probes being developed by the Jet Propulsion Laboratory (see p.6) is one obvious avenue to pursue. Another is being studied by the Space Science Board and would make the shuttle into a truly useful scientific vehicle; it is to build a module, called Spartan, that could be dumped overboard from the shuttle, allowed to co-orbit with the shuttle (unmolested by men bumping about) and then picked up just before returning to Earth.

At the same time, more care and effort should be put into the shuttle and the space station, but in terms of an engineering development project — not a crash programme to build the space vehicle for the next decade. It is of course only a matter of time before there will be some real scientific (and perhaps military) needs for a permanent space station, and plans should be made for that. Meanwhile, the United States should learn something from the Soviet Union, and not waste energy on bursts of light and heat that hurt only itself in the long run. □

The year that never was

If 1984 is not as fearsome as in Orwell's novel, there are still important battles to fight.

At the beginning of George Orwell's year, the first thing to acknowledge is that 1984 never came. The idea that governments might become so preoccupied with their own survival, and so adept at the exploitation of technology for bending the inclinations of their people to that simple goal, that personal liberty would cease to be, remains a nightmare — one, nevertheless, that will not go away. Moreover, this does not imply that governments are consistently more enlightened now than, say, a year ago, or that the suppression of liberty has by magic been banished from the face of the Earth. On the contrary, new pockets of despotism are forever cropping up. The mercy is that the practitioners of wayward offences against people are but crudely practised. Bullets, in their books, are more convenient than mind-control. And, as illiberal governments repeatedly discover, their wish to make their people toe some predetermined line is repeatedly undermined by the personal courage of those they would control. Precisely that obdurate unwillingness of people to forsake human dealings with each other is, after all, what changed Orwell's book from a political tract into a novel. Although the profession of science is superficially indifferent to the ways in which governments choose to treat their citizens, the health of science rests on the freedom with which new ideas flow from one place to another, from any person to any other. That, of course, is why professional scientists are concerned with the way in which their colleagues elsewhere are dealt with and why the fortunate among them have an obligation to assist those less fortunate by all possible legal means.

Against Orwell's predictions, the more serious anxiety during 1984 is not that governments will find it prudent to deprive their people of liberty but that they will choose to blow each other's people to smithereens. Outwardly, this again is an issue separate from the practice of science as such, or in which it might be held that professional people have a right to intervene only in their capacity as citizens. In many circumstances, that would be true. But if it is the case that the means of waging nuclear war, and the means by which the risks of nuclear warfare might be avoided, depend to a large extent on technical considerations, the technical community may be held to have an obligation to throw what light it can on the questions that arise. That is why journals such as this have always taken the position that comment on the steps which are being taken (or not being taken) to avoid the dangers of nuclear and other kinds of wars is not merely permissible but obligatory. And 1984 promises to be a busy year in this respect.

The year ahead also bristles with threats to the well-being of the scientific enterprise. The long recession is far from over (and will not be completed until the present period of rapid technological change has worked its way through the world's advanced societies). So even the most prosperous and far-sighted governments are in their different ways looking for ways of cutting costs wherever possible. The ambition is legitimate and laudable; the practice, unfortunately, is often ill-considered. This is why 1984 is likely to see a running battle between governments anxious to trim budgets by making science a pensioner of industry and those who see that the exploration and understanding of the world we live in cannot be trusted to such chance. Orwell's great book was written in 1948, when the first rudimentary electronic computers had just come into service, when the distinction between the pi and the mu mesons had just been discovered, when neither transistors nor lasers had been invented and when the mechanisms of inheritance were still unknown. That industrial needs have been the engines of many of these developments is not in question, nor is the prosperity that industrial developments have brought. The issue that will be fought in the months ahead is the extent to which free inquiry should depend on such adventitious interests. That battle too, looks like being a long and loud one. □

Intellectual property

UK patents shake-up causes a stir

A PROPOSAL to hive off the British Patent Office from the civil service and establish it as a separate statutory body is sending shock waves through the patent community. That suggestion is just one of several that are likely to prove controversial in a report on intellectual property rights recently published as a green paper*.

The report was produced at the request of the Prime Minister by Dr Robin Nicholson, Chief Scientific Adviser at the Cabinet Office, who holds that "cumbersome and slow" patent procedures are deterring would-be innovators in Britain. Smaller companies and individual inventors, in particular, tend to find the "arcane" world of the patent system a disincentive to stake a claim for patent protection. A new-look Patent Office freed from civil service constraints should, according to the green paper, be given broader terms of reference and encouraged to advertise its services and expertise, thus making fuller use of the database on patents that it maintains.

Nicholson has recently described the patent system in Britain as "dominated by a few very expert individuals" — a view apparently shared by a senior Patent Office figure who declines (for reasons of civil service etiquette) to be named but who feels the workings of the Patent Office have been partly misunderstood. And Mr Bernard Fisher, president of the Chartered Institute of Patent Agents, says that the green paper's main aim of getting the Patent Office to be more responsive to inventors' needs might be achieved more simply and more cheaply by simply allowing it to keep more of the profits it usually makes each year which are now returned to the Treasury. Pension rights and career structures would be greatly affected if the proposed changes were made, however, and allowance would have to be made for compensation payments.

The green paper urges a government policy statement on intellectual property, to include a number of changes. Some, such as the suggestion that service industries should be able to claim exclusive use of registered mark, have already been accepted by the government. Others are more contentious: the proposal to replace design copyright laws by a registered design scheme, in particular, will be unpopular with manufacturers of mass produced items, who are at present very well protected against competing manufacturers of spare parts, for example in the automobile industry. One critic of the

Nicholson report said it might be impractical for manufacturers to have to register components individually, whereas as things stand ownership of engineering drawings confers copyright protection.

Nicholson comes down in favour of a system of "petty patents" — a form of short-term patent conferring only limited protection which can be upgraded into a patent proper if the putative invention justifies the cost and effort involved. The government has previously rejected this idea but changes in practice since the introduction of the 1977 Patent Act might now have swung the balance in its favour. The complaint from some industries is likely to be that their research and development programmes would be stultified if the number of potential monopolies were increased by petty patents.

Nicholson identifies several abuses of the patent system that are not adequately checked by existing safeguards. The aim is to prevent potentially valuable innovations from being "sat upon" by patent holders unwilling to risk exploitation. The 1977 act included provisions for compulsory licences to be issued to would-be inno-

vators in such cases, but since the act was introduced only one has been granted. Nicholson says the existing provisions should be used more often and proposes new rights for employee inventors that would allow them to exploit an invention that is not taken up by their employer.

One proposal that is unlikely to be accepted without some resistance is that people other than registered patent agents should be allowed to represent inventors. The aim is to allow wider access to property rights and to promote price flexibility, but it is pointed out that inventors could lose all their rights if an inadequately prepared patent is published. The Chartered Institute of Patent Agents is apparently untroubled however, believing that serious inventors will always prefer the security of a registered agent.

One view of Nicholson's proposals is that they fail to take account of the constraints on Britain stemming from its participation in the European Patent Convention. There are certainly international issues at stake, particularly in seeking a balance between the legitimate interests of manufacturers seeking patent protection throughout the European Community, for example, and the risk of sanctioning anti-competitive practices. The document suggests that Britain should take the initiative in requesting the European Commission to devise proposals to straddle the gap.

Tim Beardsley

UK research councils

Robbing Peter to pay Paul

THE Advisory Board for the Research Councils (ABRC) has recommended that funds for restructuring the British research councils during 1985–87 should come from within the science budget, according to Mr Peter Brooke, the Under-Secretary of State for Education and Science in a parliamentary reply. This tame-sounding statement has a sting to it. It means that the Medical Research Council (MRC) and the Science and Engineering Research Council (SERC) will, over that period, forgo 0.75 per cent and 1.5 per cent respectively of their budgets to pay for the forthcoming reorganization of the Natural Environment Research Council (NERC) and the Agricultural and Food Research Council (AFRC).

AFRC has been under pressure to reorganize partly as a result of earlier ABRC recommendations to cut back on financial support for the council (see *Nature* 22 December 1983, p.723). More recently, a study carried out by Sir Ronald Mason for ABRC suggested in unspecific terms that the amount of in-house research within AFRC and NERC was excessive. Both research councils are planning reorganization in the long term which is expected to involve expenditure on "early retirements, transfer costs and capital work", ac-

cording to Mr Brooke. The science budget will be increased by £750,000 in 1984–85 and by £900,000 in the two succeeding years to offset some of this cost, while the total contributions from SERC's and MRC's forward planning budgets will amount to £3.1 million in 1984–85 and £6.3 million in 1986–87.

Mr Brooke also announced that an extra £1 million has been found partially to compensate SERC for increased subscriptions to international research organizations such as the European Organization for Nuclear Research (CERN), on top of the £6 million already secured from the Treasury. This still leaves SERC with about £3 million to find from its current budget next year.

In the circumstances, SERC and MRC are hardly likely to welcome the future loss of funds for research. Indeed, at a press conference in November, Professor John Kingman, chairman of SERC, emphasized the unfairness of a policy that penalized "efficient" councils. This reaction is apparently contradicted by Mr Brooke's statement that ABRC's policy is unanimously supported by the advisory board's members — who include the chairmen of all the research councils.

Philip Campbell

*Intellectual Property Rights and Innovation. (Cmd 9117, HMSO, £4.65).

Radiation exposure

US academy study attacked

Washington

THE National Academy of Sciences has stumbled into an uncomfortable controversy about the health of American soldiers exposed to radiation when they were stationed near Hiroshima and Nagasaki after the cities were destroyed by atomic bombs in 1945. Veterans have for years been locked in dispute with the Department of Defense about the long-term health effects of their service in the area.

Last July, in a study requested by the Pentagon's Defense Nuclear Agency, the academy's research arm, the National Research Council (NRC), published a report concluding that there was no evidence to support claims that the veterans had suffered from an abnormally high incidence of the bone cancer called multiple myeloma. The fact that the report was immediately criticized as perfunctory by the National Association of Atomic Veterans (NAAV) caused little surprise.

But now, Congress's Office of Technology Assessment (OTA) has joined the fray. In a rare instance of Congress demanding a review of an NRC study, OTA says the report did not have enough evidence for its conclusions.

The academy's involvement goes back several years. In 1981, the Defense Nuclear Agency asked whether a large-scale epidemiological study of multiple myeloma was warranted. An NRC panel under Harvard's Brian MacMahon concluded that the low level of radiation to which US troops were exposed — about a tenth of a rad — could not have been responsible for the incidence of cancer. But the MacMahon panel also said that an epidemiological study might be necessary if estimates of the level of radiation encountered by American troops were revised upward or if there appeared to be *prima facie* evidence that veterans were suffering in disproportionate numbers from the disease.

In the wake of the MacMahon report, the Pentagon asked NRC to find out what it could about the incidence of multiple myeloma. A panel set up to review all known cases of myeloma among the veterans identified only nine — at the bottom of a range from 9 to 29 expected statistically in the normal population. It concluded in July (see *Nature* 304, 200; 1983) that there was therefore no evidence to support claims of an abnormally high incidence among veterans.

OTA claims the NRC study has two basic flaws. The methods used to identify only nine cases of myeloma among the veterans were likely to result in an underestimate of the number who had actually contracted the disease. At the same time, NRC probably overestimated the number of cases that could have been expected if the rate of incidence was the same for veterans

as for the general population.

Veterans who had contracted multiple myeloma were tracked down through a nuclear veterans' "hot line" established by the Pentagon and through a newsletter and magazine article organized by NAAV. The hot line had received calls from nearly 50,000 people by the time of NRC's study, of whom 678 reported service at Hiroshima and Nagasaki, and seven of those reported having multiple myeloma. NAAV found 21 names of people who had served in the two cities and had the disease.

NRC whittled down the 28 possible cases to nine. Eleven were eliminated because they appeared not to have served in Nagasaki or Hiroshima and six because they, their families or doctor failed to respond to follow-up enquiries. In another two cases, review of the clinical evidence found that they had not, in fact, suffered from the disease.

This procedure, claims OTA, was most likely to produce an underestimate of the number of cases. For one thing, the Pentagon hot line had produced only 4 per cent of the estimated 20,000 veterans who had served in Hiroshima and Nagasaki. For another, nearly half of those who contract multiple myeloma die within the first year, and at least some bereaved relatives may have failed to respond to the hot line, particularly as the advertising did not refer to multiple myeloma. Moreover of the 11 cases eliminated because they appeared not to have been at Hiroshima or Nagasaki, only seven were definitely known to have been elsewhere. And the loss of six cases because of failure to respond to a follow-up letter and telephone call could have had a dramatic impact on the overall result.

Finally, says OTA, the report compared the number of cases expected over a 35-year period with the nine cases it had tracked down — but all nine had been reported within a five-year period. OTA claims the comparison was inappropriate; its own calculation estimated the number of expected cases to be much smaller. Taken overall, OTA concludes, NRC's approach was unlikely to have detected a disproportionate incidence of multiple myeloma even if it existed.

NRC has not yet responded to OTA's criticisms, but in the view of at least one congressman the council has some explaining to do. Paul Simon, the Illinois Democrat sponsoring a bill to compensate "atomic veterans", said NRC's report had "tainted" debate about the complex issues involved. The methods and boldness of the report were bad enough, he added; more troubling was that its conclusions agreed so neatly with the views of the Defense Nuclear Agency, which had long denied government responsibility for the health problems of atomic veterans. **Peter David**

Indian science

Research council in trouble

New Delhi

THERE are skeletons in the cupboard of India's Council of Scientific and Industrial Research (CSIR). A scrutiny by a parliamentary committee has drawn attention to several shortcomings in the functioning of India's leading industrial research organization. The committee complains in particular that very few processes developed by the CSIR laboratories have been found useful by industry.

Only recently, Prime Minister Indira Gandhi was forthright in her criticism of the laboratories on the grounds that their research projects incur substantial losses. At a meeting of directors of national laboratories, she said that these are becoming an "ever-increasing burden on the nation" and asked that if there is no hope of the situation improving, "is it not time just to close them down?"

Now the parliamentary Public Accounts Committee (PAC) has revealed what ails these laboratories. Research projects have often been taken on without adequate planning in advance. The lack of machinery, equipment and other necessary facilities has later led to the abandonment of many such projects. In the past two years, about 300 projects have been dropped, reducing the number of schemes in hand to 1,543. A further hundred are to be terminated next year.

The officials concerned in evaluating these projects display a "superficial and perfunctory" attitude, according to PAC, which says that while unproductive and unfeasible projects should normally be abandoned after one year, some of them were allowed to continue for as long as five years. A glaring example is that of a household pump research project which had to be discarded after two years of work when it was found that the same project had been undertaken by another laboratory of CSIR three years earlier.

PAC has remarked that either the processes developed by CSIR were not selected with the needs of Indian industry in mind or CSIR has been unable to inspire the confidence of potential users in the usefulness of its processes. The council referred 295 of its technical development projects, less than half of those on its books, to the National Research Development Corporation (NRDC) for industrial exploitation, of which only 15 per cent had been taken up by industry by June 1981 — and some of these were withdrawn by CSIR.

Even public sector undertakings are not making full use of the council's research output. CSIR has been complaining of an "unsympathetic attitude towards development and utilization of indigenous know-how", saying that many of its

schemes are exposed to unfair competition "by the import of technology from abroad". It has been demanding a role in the selection of technology imports. It ranks that last year, CSIR technology for colour television was not exploited but that, instead, the government allowed the import of colour television sets.

The director general of CSIR agrees with the criticism that the council's laboratories concentrate on projects which serve the elite sections of society and pay little attention to technologies that could benefit people in economically weaker sections of the community. It has accordingly compiled a list of technologies to be developed for poor people.

Younger scientists have found support

from PAC for their complaint that their views are ignored in planning the research work of laboratories. The committee says that funds allotted to their work are often arbitrarily transferred to other projects sponsored by more senior scientists, while younger people tend not to be included in the high-level committees of different laboratories.

CSIR is concerned that it can no longer attract scientists of high calibre for senior posts, partly because its laboratories are poorly equipped. One of its difficulties is the rapid inflation in the prices of chemical apparatus, equipment, books and journals, so that even a 10 per cent increase in its budget is not enough to catch up.

Sunil Saraf

Animal rights

Massachusetts bans strays

Boston

THE giving or selling of dogs and cats from city pounds and animal shelters to laboratories has been outlawed by the Massachusetts legislature. Responding to overwhelming public support for the measure, the bill has now been signed by Governor Michael S. Dukakis.

Animal rights advocates are claiming a major victory, as Massachusetts becomes the eighth state to prohibit pound seizure — the right of licensed laboratories to purchase unclaimed animals after 10 days. The law will end pound seizure from next October and the purchase of dogs and cats imported from out-of-state pounds from two years after that. Massachusetts is the first state to enact this provision.

Researchers who depend on so-called "random source" dogs and cats will now have to buy more costly "purpose-bred" animals from commercial breeders, or switch to other experimental subjects such as sheep, pigs, calves and goats. Ralph Charwood, assistant director of the Harvard Animal Resource Center, says that the law will dramatically increase costs for heart, kidney and toxicology research that now depends on pound animals. Boston area medical schools are considering solving the problem of supply by establishing a jointly operated animal breeding facility, but even this approach would be more costly than buying cats and dogs from pounds.

Walter Kilroy, vice-president of the Massachusetts Society for the Prevention of Cruelty to Animals (MSPCA), claims that the law will improve the quality of research. "Random source animals are not good research models", he said. "Their genetic background and physical condition are much more variable than bred animals." Opponents of pound seizure believe that domesticated dogs from pounds suffer more stress than those used to kennel conditions and maintenance. "Laboratories taking dogs from pounds accept only those animals with person-

alities that allow them to be handled and restrained", said Kilroy. These are pets, accustomed to "human companionship and affection, and liberty".

Research institutions in Massachusetts — Harvard University and the University of Massachusetts in particular — have for several years been fighting highly organized efforts to repeal the pound laws; state legislators receive more mail on this issue than any other. ProPets, an alliance between the New England Anti-Vivisectionist Society (NEAVS) and MSPCA, collected more than twice the required number of signatures on a petition to put on this year's ballot a referendum far more



restrictive than the law now to be enacted. Unwilling to face the huge costs of fighting a state-wide referendum, the research institutions agreed to the new legislation in a compromise with ProPets hammered out by State Representative Ray Jordan.

Legislative efforts to prohibit pound seizure appear to be gaining momentum in other states. Aaron Madlock, director of the New England Anti-Vivisectionist Society, says his group will now campaign for similar legislation in Vermont, and points out that animal rights groups in California are engaged in a major battle to ban both pound seizure and importation from other states. Although universities, medical schools and pharmaceutical companies are now attempting to persuade the public of the value of animal research, they have plenty of catching up to do.

Christopher Earl

Soviet oceanology

Institute director in deep water

DR Andrei Sergeevich Monin, director of the Institute of Oceanology of the Soviet Academy of Sciences, has recently come under sharp attack in the Soviet media and from Communist Party activists within the academy. Charges against Dr Monin range from "the embezzlement of socialist property" (having his dacha repaired by institute employees and out of institute funds) to despatching research ships "round the world and back again" without a research programme for the whole journey, and from failing to ensure a "proper moral and political climate" in the institute's oceanological equipment design bureau (which is said to be run by a "close relative" of Dr Monin) to applying for the right to search for sunken treasure. Moreover, it is claimed, he has consistently ignored the criticisms of local and academy party officials, and has attempted to dismiss from the institute anyone who complained too openly of his policy.

As far as the corruption charges are concerned, the institute appears to have been made an example in Mr Andropov's much-publicized campaign for "work discipline". Unauthorized disbursements from the bonus fund and discreet "rectification" of over-demanding plans have become, alas, all too common a feature of Soviet management — including the management of scientific institutions. According to *Sovetskaya Rossiya*, several employees of the Institute of Oceanology's Leningrad branch have already been brought to trial on charges of "fraud and currency machinations". Nevertheless, it is alleged, the deputy director, L.A. Tzymbal, who was discovered to have sanctioned and received illegal salary increments, is still working at the institute under Dr Monin's patronage.

Dr Monin has, apparently, so far managed to quash all complaints against him, including those made to the academy. Letters of complaint get bogged down in the bureaucratic process or else receive smooth and non-committal answers. Several leading oceanologists have allegedly left the institute in disgust at the director's seeming inviolability. Meanwhile, Dr Monin himself has a standard answer to every criticism: it is all due, he says, to the "growing pains" of the relatively young science of oceanology.

Vera Rich

Correction

The accidental sea discharge at Sellafield reported in *Nature* (306, 721; 1983) was made through the normal sea pipeline, and not as stated. The 2-inch pipe was used in an attempt to return waste from the sea tank to the plant. □

US space plans

Economy flights in prospect

Pasadena, California

CUT-rate planetary missions have been included in the 1985 budget request of the National Aeronautics and Space Administration (NASA). The series of probes, known as "Observer", will use modified Earth-orbital satellites to explore the nearby planets. The first flight could take place in 1982, with subsequent missions at one to two year intervals.

The new probes would add significantly to the modest resurgence in planetary exploration of the past year. Congress this fall approved funds for the Venus Radar Mapper mission, the first new planetary mission since Galileo. Galileo, which was to have been launched on its journey to Jupiter in 1982, was repeatedly delayed by setbacks in the shuttle programme, and is now scheduled for 1986.

Costs for the Observer probes will be kept down by designing instruments to fit available vehicles, rather than the other way around as in past practice. The Jet Propulsion Laboratory (JPL) here, which has primary responsibility for planetary missions, is ready to issue a request for proposals from satellite manufacturers for supplying the vehicles. Possible candidates include the RCA weather and communications satellites, TRW's satcoms, and Hughes communication satellites. By limiting the Observer missions to the nearby planets, Mars and Venus, requirements for power, temperature control and communications systems will be modest and can easily be adapted to fit the adapted Earth-orbit satellites. One of the first Observers is expected to be a Mars orbiter that will probe the geochemistry and climate of that planet.

The renewed hopes for planetary science come just two years after JPL, finding it hard to keep its staff employed, sought and received unprecedented permission from California Institute of Technology (which operates JPL for NASA) to take on defence contract work in an amount up to one-third of the laboratory's total operating budget. But with things looking up (besides the Venus Radar Mapper and the hoped-for Observers, JPL is busy with Galileo and the forthcoming rendezvous of Voyager with Uranus and Neptune), the laboratory expects to hold the defence work to its present level of 20 per cent.

JPL also has plans for more ambitious planetary missions. So-called Mariner Mark II probes, which similarly would draw on existing hardware designs, are being planned for exploration of more distant planets and perhaps comets; one much discussed idea would be a Saturn orbiter that would carry an entry probe to be dropped into Titan, Saturn's volcanic moon. Both the Observer and Mariner Mark II flights could be carried out under a \$300 million annual budget, according to

JPL officials. The Mariner Mark II probes would be launched every two to three years.

Much less is being heard about grand, novel missions, such as a sample-return mission to Mars. JPL officials see those as "augmentation" missions that will only be contemplated if extra money becomes available.

Stephen Budiansky

Soviet space programme

Not without hitches

AFTER more than three months of Western speculation, Soviet cosmonauts Vladimir Lyakhov and Aleksandr Aleksandrov have at last admitted that during their 150-day mission aboard the Salyut-7/Soyuz-T9 complex, there had indeed been a "slight" leak of propellant, although, they said, this "had not affected either the flight or the research programme".

Ironically, on the same day that the cosmonauts talked about their flight at a press conference in Moscow, *Aviation Week and Space Technology* published further claims about the Lyakhov-Aleksandrov mission. During their final weeks aboard the station, it was claimed, the cosmonauts had had to endure "cold, damp and uncomfortable conditions" due to a "solar array problem", that had "reduced electrical power and seriously affected" the environmental control system. The crew's two space walks on 1 and 3 November, *Aviation Week* said, had been necessary to install additional solar panels, in order to save the station from becoming uninhabitable in the near future. When the loss of environmental control became serious, *Aviation Week* claims, the Soviet planners decided to "train and launch" a repair crew — cosmonauts Vladimir Titov and Gennadii Strekalov. When the launch had to be aborted because of a fire in the booster-rocket, however, Lyakhov and Aleksandrov had to carry out emergency repairs themselves.

According to the Soviet flight controllers and the cosmonauts themselves, however, the work on the solar panels had always been "one of the most responsible parts" of the original flight programme. The new panels were needed, they explained, to increase the power supply of Salyut-7 so that more experiments could be performed simultaneously. Deployment of the panels, moreover, had confirmed that the techniques and procedures proposed for large-scale assembly work in space, are, indeed, practicable. It also provided data for evaluating and improving the ground-level training programme for such assembly work in space as well as useful feedback for the designers of space-suits.

French get hooked

JUST before Christmas the French post office, PTT, inaugurated "Minitel", an electronic telephone directory designed to lead the French market into the Elysian Fields of the "hooked-up home".

Minitel has been on trial in Brittany and Versailles, but now the experiment has been extended into part of Paris. By March, there should be 50,000 sets operational in the centre of France, and 3 million should be in action by 1986.

Basically, Minitel is designed to replace paper directories: a user dials 11 to link up to a local computer, and then — using a keyboard — answers a few questions to tell the computer the name (and/or address) whose number is sought. The answer is displayed on a small television screen. But of course, with such technology in place many other uses spring to mind — one option is screen-to-screen communication between two Minitels, so the deaf can use the telephone.

In Velizy-Versailles PTT has offered other services: banking, cinema booking, rail timetables, stock exchange prices, even a newspaper; and the take-up has been moderate. One-third of the Minitel owners (who like the others in the experiments have been given them free, though those outside experimental areas can rent them at £6 a month) do not use the machines at all; but the other two thirds use them an average of 40 minutes a week. The biggest users are those with children between 12 and 15; the smallest, retired people.

Where people have been offered the choice of a Minitel or a conventional directory, 46 per cent have chosen Minitel. It is nearly the figure forecast by PTT (50 per cent), but not enough yet to encourage PTT to abandon its printing machines.

Robert Walgate

The Soviet claim that the deployment of the panels was from the beginning an integral part of the flight programme accords with recent hints that the construction of a permanently-staffed orbital station (always a long-term aim of the Soviet space programme) is now a definite project. Moreover, if it were simply an emergency repair job, carried out in the absence of the trained "repair team", it is remarkable that Lyakhov and Aleksandrov could carry it out without, apparently, undue difficulty. According to their own account, however, the cosmonauts had carefully practised the whole procedure (in the simulation tank) and the flight-plan had been worked out so that the main operations were carried out within the radio-visibility zone and in the illuminated part of the orbit. The special equipment, moreover, used in deploying the panels included not only tools and manipulators, but also non-slip safety handrails — once again, substantiating the Soviet claim that this was a pre-planned "experiment", not an emergency repair job.

Vera Rich

Smallpox eradication

Military use of vaccine criticized

Washington

NEARLY four years after smallpox was universally eradicated, the US military is still administering the vaccine as a "defensive and deterrent measure" against the use of smallpox virus as a biological weapon. This policy has put the US Army indirectly at odds with the World Health Organization and the US Centers for Disease Control (CDC), both of which have been working to eliminate all use of the vaccine. 'We've had several deaths and significant morbidity from its use for non-indicated reasons', says Dr Stanley Foster, assistant director of CDC's international health programme office. The main civilian users have been physicians who either did not know that smallpox had been wiped out or who believed that smallpox vaccine was effective against genital herpes. CDC last spring persuaded Wyeth Laboratories, the only remaining manufacturer of the vaccine in the United States, to take the product off the commercial market. Wyeth had at last count been selling some 50,000 doses per year.

Wyeth is, however, continuing to supply the military. All active duty personnel and reserves are vaccinated on a five-year schedule — which amounts to several hundred thousand doses per year. The military has also requested Wyeth to supply a stockpile of the vaccine sufficient to dose a classified number of personnel in the case of a general call-up.

Adverse reactions to the vaccine, which contains live vaccinia virus, are rare, but not insignificant. According to CDC, one death can be expected for every million

persons vaccinated. Generally less severe reactions (although sometimes fatal themselves) occur more frequently, mostly in certain susceptible groups — immunocompromised subjects (about 7 reactions per million vaccinations) and those with eczema (38 per million).

Dr James Kirkpatrick, an army lieutenant colonel who advises the Army Surgeon General's office on the smallpox vaccination programme, says that by exempting personnel with eczema (or who have a family member with eczema), the military has kept the rate of adverse reactions quite low. All vaccinations (including measles, rubella, tetanus, smallpox and a half dozen others) resulted in 60 hospital admissions a year, he said, with an average hospital stay of two days. Although no numbers are available for the rate of adverse reactions specifically from smallpox vaccinations, CDC says it receives about one request per month for vaccinia immune globulin, which is used to treat reactions to the vaccine. All such requests since last spring have been from the military.

The military's reason for vaccinating against smallpox appears to be intelligence information that the Warsaw Pact nations and the People's Republic of China vaccinate their troops. 'Nobody can rule out the possibility' that smallpox would be used as a weapon, Kirkpatrick says, and it 'would not be prudent' to stop vaccinating. According to CDC, only the United States and the Soviet Union continue to maintain stocks of the smallpox virus. South Africa recently and with much fanfare announced the destruc-

Richter to head SLAC

Washington

BURTON Richter has been named to succeed Wolfgang Panofsky as director of the Stanford Linear Accelerator Center (SLAC). Richter, who won the Nobel Prize for Physics in 1976 (with C.C. Ting) for the discovery of the psi particle, will take up the post next September. Richter is the designer and prime mover of SLAC's linear collider, on which construction began two months ago. The \$113 million machine consists of a one-and-a-half mile loop attached to the main linear accelerator; electrons and positrons accelerated in the linear stretch will collide in the loop at centre-of-mass energies of up to 100 GeV. The linear collider, like the much larger LEP machine under construction at the European Organisation for Nuclear Research (CERN) at Geneva, will find its greatest application in detailed studies of the Z^0 particle. The electron-positron collisions are much cleaner and easier to interpret than the proton-antiproton collision used at CERN last year to detect the heavy bosons (Z^0 and W^+ / W^-). Unlike LEP, however, the linear collider will be breaking new ground in accelerator design — a design that Richter says will yield considerable cost savings over LEP's traditional ring structure.

Panofsky, who has directed SLAC since 1961, decided to follow his own policy of retirement at the age of 65 for the laboratory's administrators but will act as a technical adviser. Stephen Budiansky

tion of its stocks.

Although CDC has been careful not to criticize the military programme directly, CDC officials are known to be uneasy. And a number of smallpox researchers have questioned whether the supposed threat of smallpox as a biological weapon outweighs the known risks of vaccination. Smallpox has an 8–10 day incubation period and is not very infectious. Epidemiologic evidence shows that 30–40 per cent of susceptible members of a household will catch the disease from an infected member, compared with 80–90 per cent for measles, for example. They also point out that smallpox virus would live only a matter of hours in an aerosol form, the presumed method of delivery as a weapon.

The use of smallpox vaccine by civilian physicians as an unorthodox treatment for genital herpes has been a bizarre footnote to the story. Controlled studies have demonstrated no benefit in preventing or healing herpes lesions; CDC has nonetheless received many calls from herpes patients protesting against CDC's role in removing the vaccine from the market and claiming that the vaccine was the only thing that had helped them. CDC officials say that some physicians had also apparently been using the vaccine as a general "tonic", supposedly to boost a patient's immune defences.

Stephen Budiansky

US dioxin question is tackled

Washington

A MAJOR new programme to deal with dioxin has been unveiled by the Environmental Protection Agency (EPA), fulfilling a promise made by William Ruckelshaus when he became its administrator last spring. The programme will include investigations at hundreds of chemical plants and waste disposal sites at a cost of up to \$250 million over four years. Alvin Alm, EPA's deputy administrator, says the agency regards dioxin as one of the most perplexing and potentially dangerous chemicals ever to pollute the environment. The programme would concentrate on what was considered the most toxic of the 75 dioxin isomers — 2,3,7,8-TCDD, which is known to cause chloracne in humans. In laboratory animals, it has been known to cause cancer, reproductive failure, reduced effectiveness of the immune system and significant changes in enzyme systems. EPA's Cancer Assessment Group says it should be regarded as both an initiator and a promoter of cancer.

The agency's top priority will be an investigation of sites at which the herbicide and feedstock chemical 2,4,5-TCP was manufactured or disposed of. The chemical was used in the manufacture of 2,4,5-T and silvex, well-known herbicides widely used until recently to control weeds and as defoliants. 2,4,5-T was an ingredient of Agent Orange, the defoliant used in Vietnam. In a background document, EPA admits that little is known about the effect of dioxin on humans. A single dose of less than a millionth of a gram can kill a guinea pig, but its potency varies enormously between species. Information on the effects of human exposure is scarce. It is agreed that dioxin can cause chloracne but it has not been proved that it can also cause soft tissue sarcoma.

The uncertainty has resulted in a chequered history of regulation, and in October last year the Dow Chemical Company voluntarily cancelled its registration of the products.

Peter David

Releasing "ice-minus" bacteria

SIR — Two strategies for the biological control of those bacteria that act as seeds for ice-crystal formation (and the consequent crop damage) have recently been presented. Researchers at the University of Colorado propose spraying fields with bacterial viruses (bacteriophage)¹, while researchers at the University of California propose spraying fields with genetically engineered bacteria whose "ice-nucleation gene" has been deleted^{1,2}. Most of the media attention has gone to the latter stratagem, largely because of the controversy surrounding the release of recombinant DNA organisms into the environment^{2,3}. However, successful implementation of these strategies may be limited by ecological and evolutionary factors subsequent to the deployment of predators (bacteriophage) or competitors (recombinant bacteria). The application of bacteriophage to populations of sensitive bacteria engenders intense selection for phage-resistant mutants⁴ whose increase could defeat future, if not present, control. The application of recombinant "ice-minus" bacteria requires that they out-compete naturally occurring ice-nucleating forms², whereas the arbitrary deletion of an evolved gene would likely be disadvantageous, or at best neutral.

An integrated strategy might overcome these difficulties, and might even begin to counter some of the fears associated with releasing recombinant bacteria. Bacteriophage infect bacterial cells by first adsorbing to some surface protein or other component of the cell wall; phage are generally very specific in their site of adsorption⁵. By comparing the sensitivities of "ice-minus" and "ice-plus" isogenic bacteria to a diverse set of bacteriophage, one might identify a phage whose site of adsorption is the ice-nucleating surface protein. By applying this bacteriophage in concert with the "ice-minus" recombinant bacteria, one would provide the "ice-minus" phage-resistant bacterium with a strong selective advantage over the "ice-plus" phage-sensitive natural counterpart. Moreover, as phage-resistant mutants appeared among the naturally occurring bacteria, they too would probably be "ice-minus" since resistance to phage typically arises via the alteration or loss of function of the gene encoding the specific surface protein to which the phage adsorbs⁶; even if such phage-resistant mutants were not "ice-minus", selection favouring their increase would be ameliorated by high densities of the phage-resistant recombinant competitor. Finally, with this strategy, it should be possible to release "disarmed" strains of "ice-minus" recombinant bacteria — recombinant strains bearing genes (or deletions of genes) which ensure their competitive inferiority outside the range of simultaneous application of bacteriophage, yet whose phage-resistance

renders them selectively favoured within the range of simultaneous phage application. Thus, the spread of recombinant bacteria would be prevented by virtue of their competitive inferiority coupled with their inability to support the very phage whose presence is necessary to permit their establishment.

Is there a precedent for this strategy? Williams Smith and Huggins⁶ successfully treated experimental bacterial infections in mice using bacteriophage, precluding the rise of phage-resistant bacterial mutants in a manner similar to that proposed above. First, pathogenicity of the target bacterium was shown to depend on the presence of a particular surface antigen⁷.

Second, a bacteriophage was isolated whose site of adsorption is that surface antigen. Third, it was shown that the bacteria became resistant to the phage through the loss of that surface antigen. In essence, Williams Smith and Huggins chose phage for which resistance was incompatible with pathogenicity of the target bacterium. Whether phage can be isolated for which resistance is incompatible with ice-nucleation by the target bacteria remains to be seen.

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1. Miller, J.A. *Science News* 124, 132 (1983).
2. David, P. *Nature* 305, 262 (1983).
3. Budiansky, S. *Nature* 305, 564 (1983).
4. Levin, B.R. & Lenski, R.E. in *Coevolution* (eds Futuyma, D.J. & Slarkin, M.) 99–127 (Sinauer, Sunderland, Massachusetts, 1983).
5. Schwartz, M. in *Virus Receptors Part 1, Bacterial Viruses* (eds Randall, L.L. & Philipson, L.) 59–94 (Chapman and Hall, London, 1980).
6. Williams Smith, H. & Huggins, M.B. *J. gen. Microbiol.* 128, 307 (1982).
7. Williams Smith, H. & Huggins, M.B. *J. gen. Microbiol.* 121, 387 (1980).

How "gradual"?

SIR — The recent article by Rhodes¹ on "Gradualism, punctuated equilibrium and the *Origin of Species*" is a major event in clarifying Darwin's views on both rates of evolution and "gradualism". The claim that "gradualism" refers specifically to long-term regular changes in the geological record² has led to much confusion. It is now clear¹ that Darwin's views on gradualism should be understood on a shorter biological or ecological time scale³, and that they do not require (nor could they explain) slow, long-term, orthogenetic changes in the fossil record.

There is one point that has not been covered and which helps to establish how (apart from misquotation³) the misunderstanding arose. It may also help to prevent such errors in future. This is that terms such as "fast", "slow" and particularly "gradual" have no scientific relevance unless they are used with respect to a specified time scale.

The term "temporal solipsism" has been

used by Kamen⁴ to describe the confusion that arises when conclusions are applied to the wrong time scale. His example is photosynthesis and he pointed out that it can be studied over many different time scales. Radiation physicists study quantum processes that occur within 10^{-15} – 10^{-9} seconds, or even longer. This "era" extends over at least six orders of magnitude, and overlaps with the time frame of photochemistry which studies events from 10^{-9} to about 10^{-4} or 10^{-3} s. Biochemists study events occurring from about 10^{-3} up to 10 s, and physiologists extend the range up to 10⁴s. Crop physiologists, farmers and ecologists extend the domain to at least 10⁵s. But long-term evolutionary changes such as the relative sensitivity of carboxylating enzymes to CO₂ and O₂ (ref. 5), development of C₄ photosynthesis and crassulacean acid metabolism, extend into the domain of more than 10¹⁶s.

Given this extraordinary range of 10^{-15} – 10^{16} s, it is clear that "fast", "slow" and "gradual" are meaningless unless a temporal frame of reference is given. The interpretation suggested is that some palaeontologists have shown "temporal solipsism" by assuming that gradualism referred specifically to a geological time scale. It is clear^{1,3} that Darwin, in writing a book for a general readership, was referring to a biological or ecological time scale^{1,3} in discussing small, gradual changes between generations. Such a process does not predict orthogenetic trends in the fossil record.

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1. Rhodes, F.H.T. *Nature* 305, 269 (1983).
2. Eldredge, N. & Gould, S.J. in *Models on Palaeontology* (ed. Schopf, T.J.M.) 82–115 (Freeman, Cooper, San Francisco, 1972).
3. Penny, D. *Syst. Zool.* 32, 72 (1983).
4. Kamen, M.D. *Primary Processes in Photosynthesis* (Academic, New York, 1963).
5. Miziorko, H.M. & Lorimer, G.H. *A. Rev. Biochem.* 52, 507 (1983).

Be kind to flies

SIR — E.G. Gray's method for swatting flies¹ may work² but must always result in the death of the fly which cannot be justified unless the insect in question really is a health hazard. Last Christmas I bought my arachniphobe family a simple device called a "Spider Scoop". This consists of a transparent plastic dome mounted scissor-like on a broad plastic base. The dome is put over the offending arthropod and the base gently brought into place trapping the animal inside it. The principle resembles that of the card and beaker technique³. I recommend this method of fly trapping as efficient, clean and kind.

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1. Gray, E.G. *Nature* 304, 678 (1983).
2. Miller, A. *Nature* 305, 664 (1983).
3. Kruthoff, P.N. *Nature* 305, 570 (1983).

New window on Tibetan tectonics

The latest reports from the Sino-French expedition in Tibet show a rich and surprising harvest of discovery. Has not the time come for more open access to this important region?

TIBET has always been a mysterious place. In the nineteenth century, wide-eyed explorers wrote of the region with an innocence unclouded by the circumstance that Marco Polo had passed that way some centuries earlier. Physical isolation has all along been enhanced by human geography, and in particular by the self-imposed isolation of the Tibetan people, broken chiefly by expeditions bent on climbing Mount Everest. The consequence is that very little has been learned at first hand of the most rudimentary geological characteristics of this remarkable region and that many of the attempts in the past few years to relate the tectonic structure Tibet (and much of the rest of central Asia) to the tectonic collision of the Indian subcontinent with the Asian land-mass have had to rely to an unhealthy degree on inferences from topographical features.

Now, at last, there is a good chance that some of these uncertainties will be removed. The group of articles appearing on pages 17–36 is the second instalment of the fruits of the Sino-French collaboration on the geology of Tibet (see also *Nature* 294, 405–417; 1981). In two successive seasons (1980 and 1981), modern techniques have for the first time been applied to the study of the gross tectonic structure of Tibet. By means of seismic records from artificial explosions, it has in particular been possible to map beneath Tibet the Mohorovicic discontinuity which is probably the best indicator of the continental basement. The result is both important and surprising.

That the collision of the Indian subcontinent with the Asian underbelly must have had a dominating influence on the structure of central Asia has been evident since it was first recognized that the Indian subcontinent traversed the whole length of the Indian Ocean from about 50 million years ago until its collision with Asia rather more than 20 million years ago. The join between the two continents is recognizable in the long east-west suture along part of which the Brahmaputra flows eastwards, north of the Himalayas. The Himalayas themselves are explicable as tectonic consequences of the collision between two land-masses — formed from material uplifted from the northern leading edge of the Indian subcontinent and partly metamorphosed in the process.

In recent years, the bigger puzzle has been that of understanding what may have happened north of the suture, beneath

Tibet itself. The great elevation of central Asia suggests that an ancient continental crust has been thickened, while the predominantly east-west alignment of major features between the Caspian Sea in the west and mainland China in the east suggests crustal shortening in a north-South direction and extension at right angles. Whether the elevation of central Asia might be explained by the addition of Indian material to the underlying basement, almost as if the southern continent were sliding beneath Asia, has been a lively issue amongst geophysicists.

What now emerges from the Sino-French study of Tibet along two north-south traverses is that the simple picture of a once-and-for-all collision is too simple. The most striking result of the data gathered by the 1981 expedition is that there is a second suture in the tectonic structure of Tibet which, on the face of things, is in every way comparable with the southern suture but some hundreds of kilometres further north. The inference is that the collision of India with the underside of Asia was preceded some tens of millions of years earlier by the collision of another continental fragment with the same region. And that layer of material has been thickened, and elongated from the east to west, between the anvil of the main body of Asia to the north and the still-moving hammer of India, now shown to be moving relative to the Tibetan blocks at about 5 centimetres a year.

The evidence for this new view of the recent history of south-central Asia is impressive. Palaeomagnetic measurements show that the Lhasa block, the first tectonic structure north of the Himalayas, was sited at or south of the Equator in Cretaceous times. In the region covered by the Sino-French surveys, the continental basement is a patchwork suggesting that the high plateau of central Asia is made up not simply of two fragments (the Indian subcontinent and its predecessor) but of several. And there is evidence to suggest just how relics of past continental fragments underline each other, contributing to the thickening of the continental crust throughout this region.

For those with an interest in the evolution of Gondwanaland, these developments must be portentous. On the simplest reconstructions, Gondwanaland is an amalgam of those continents lying on or south of the Equator. Both the manner of their assembly into a larger continent

and the timing of their dispersal are reasonably well catalogued. But the reconstruction of the supposedly earlier super-continent in which the northern land-masses are also combined has always been more speculative and less certain.

If now it is clear that the shapes of many of these structures have been distorted by later tectonic events, the awkwardness of more ambitious reconstructions will be better understood. But naturally, the question will be sharpened why a whole series of continental fragments should have been sent scurrying north across the Indian Ocean in the past few hundred million years. Why in that direction and not some other? Or was the assembly of southern Asia an even more complicated process than it now appears to have been?

For others, the work now published is still not enough to satisfy legitimate curiosity about this geologically important region. Moreover, it is clear that what has been accomplished in a few short seasons of work with modern equipment is merely a scratching of the surface. In an ideal world, there would now follow a systematic programme of exploration designed to amass the details on which accurate interpretations can alone be based. (Why, in any case, should there have been a single land mass in the early Cambrian?)

The obvious snag is that Tibet is almost as inaccessible now as in the days before its annexation by the People's Republic of China. The collaboration with the French is the only significant opening to the outside world so far. In everybody's interests, those of the people of China especially, there need to be many more. And readers of the articles now published will be struck by the irony that the 1981 Sino-French expedition was prevented for administrative reasons from continuing north beyond Tibet into China proper. The time has surely come when such restrictions should be swept away.

John Maddox

Sir Charles Frank

IN an article on snowflakes in *Nature* on 3 November (306, 13; 1983), it was erroneously stated that Professor F.C. Frank (whose name was mis-spelled) of the University of Bristol had died in 1982. *Nature* is glad to report that this is not the case and wishes to apologize to Sir Charles, his friends and colleagues and its other readers for this embarrassing mistake. □

Neurological disease

Of Mice and Men

from J. B. Martin

SPONTANEOUS autosomal recessive mutations in mice are providing novel neurobiological models for studies of disorders of the central and peripheral nervous systems¹. The clinical syndromes observed in such mice are given graphic anthropomorphic names: weaver, shiverer, wobbler, totterer, reeler, twitcher, and so on. Such mutants are being carefully investigated in search of clues to the pathogenesis of their disorders, both to gain an understanding of normal development of the brain and to seek hints to the nature of genetic disorders that affect the human central nervous system. Anatomic and biochemical studies of each mutant type have revealed some interesting abnormalities in brain, spinal cord or peripheral nerve. In general, homozygotes are severely affected; heterozygotes less so. Two such mutants have recently been the subject of new biochemical and anatomical investigations. In the shiverer mouse, which fails to synthesize myelin basic protein (MBP) — the most important constituent of the sheath of central nervous system axons — the defect has now been identified as a mutation in the structural gene for the protein². And weaver mice, best known for cerebellar abnormalities, are, in a paper published in this issue of *Nature*, shown for the first time to have a highly selective degenerative disorder of subcortical dopaminergic systems somewhat reminiscent of certain human neurological disorders⁴.

The brains of homozygous shiverer mice contain less than one per cent of normal levels of MBP; heterozygotes contain about 50 per cent, indicating a gene dose effect. Demyelination is prominent throughout the CNS and is believed to be a contributory cause of the clinical symptoms.

The recent work by Roach and co-workers² is the first successful attempt to relate a structural protein defect to an abnormal gene in mutant mice with neurological syndromes. Working from the known structures of mice and rat MBP, which share close homology³, the investigators synthesized DNA probes based on the reverse translation of the amino acid sequence of rat MBP. Two cDNA clones encoding MBP were selected and a restriction enzyme fragment from one was completely sequenced. When translated, a portion of this sequence was identical at 126 of 127 positions with the known amino acid sequence for small MBP in the rat. Brains of shiverer mice homozygous for the MBP defect had greatly reduced concentrations of MBP mRNA compared to wild type. With the use of Southern blots for genomic DNA, it was shown that MBP

sequences are deleted in shiverer mice. These studies have considerable implications for the investigation of demyelinating diseases in man. Multiple sclerosis, though usually a sporadic not an inherited disorder, has an increased incidence in individuals with certain tissue types (HLA-A3, B7, Dw2) suggesting a genetic propensity for the disorder, at least in some individuals. More important, the fact that rodent (rat and mice) MBP is closely similar in structure to human MBP points to the potential advantage of using such animal models to investigate human afflictions. It is likely that an understanding of the gene transcription and post-translational processing of MBP will increase our understanding of the aberrations of this important protein in the human.

In this issue of *Nature*, Roffler-Tarlov and Graybiel⁴ describe an intriguing biochemical and anatomic study of the dopamine abnormality known to occur in the brain of the weaver mutant. Previous studies of this mouse focused on the cerebellum where abnormal migration and death of granule cells were noted⁵. The weaver strain also has a partial deficiency of dopamine in the forebrain⁶. The important contribution of the new report is the demonstration that one dopamine system, the nigrostriatal, is affected, whereas the other, the mesolimbic, is normal. The biochemical deficiency of dopamine in the putamen and caudate is mirrored by an alteration in the specific histofluorescence of the amine-containing neuronal elements. The dorsal striatum, which has a rich innervation from the nigrostriatal tract, shows dopamine depletion, whereas the ventromedial striatum and the nucleus accumbens, innervated by the mesolimbic system, are normal. The authors point to a lack, at the moment, of a similar disorder in humans but make analogies between this genetic disorder and the pattern of cell degeneration in Huntington's disease, an autosomal dominant human disease, recently shown to be associated with a DNA polymorphism on chromosome 4 (see refs 6 and 7). The regional distribution of the cell death that occurs in Huntington's disease is similar. Specifically, the area of the basal ganglia most affected in Huntington's disease is the dorsal lateral region of the caudate and putamen (neostriatum). In contrast, the substriatum, in particular the nucleus accumbens, is relatively spared⁸. The possibility of discovering genetic models of disease with topographic features similar to those present in man is raised by these observations.

The defect in the weaver mouse more

closely approximates that of Parkinson's disease, which is usually a sporadic illness, but occasionally occurs as a hereditary or familial disease. The exact nature of the cellular pathology responsible for the dopamine deficiency in the weaver mouse has yet to be defined, and its genetic basis is unknown.

Mutants may also be useful models for exploring new therapeutic approaches. When grafted nerves of twitcher mice, which are defective in the enzyme galactosylceramidase (a model of Krabbe's disease), are transplanted into normal mice, the enzyme defect is corrected after several weeks⁹. Enzyme replacement occurs from adjacent normal tissues pointing to a possible strategy for correcting enzymatic defects. It is likely that future studies of mice mutants will provide additional examples of nature's errors which are applicable to an understanding of human diseases. In search of these, it is evident that defined biochemical changes rather than descriptive anatomic studies will provide the important leads to follow. □

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1. Baumann, N. *Neurological Mutations Affecting Myelination*. (Elsevier/North-Holland Biomedical, Amsterdam, 1980).
2. Roach, A., Boylan, K., Horvath, S., Prusiner, S.B. & Hood, L.E. *Cell* **34**, 799 (1983).
3. Martenson, R.E. In *Biochemistry of Brain* (ed. Kumar, E.) 49-74 (Pergamon, New York, 1980).
4. Roffler-Tarlov, S. & Graybiel, A.M. *Nature* **307**, 63 (1984).
5. Sidman, R.L. in *Physiological and Biochemical Aspects of Nervous Integration* (ed. Carlson, E.D.) 164-193 (Prentice-Hall Inc., Englewood Cliffs, 1968).
6. Lane, J.D., Nade, N.S., McBride, W.J., Aprison, M.N. & Kusano, K. *J. Neurochem* **29**, 349 (1977).
7. Gusella, J. et al., *Nature*, **306**, 234-238 (1983).
8. Von Sattel, J.-P., Ferrante, R.J. & Richardson, E.P. *J. Neuropath. and Exp. Neurol.* (in preparation).
9. Scaravilli, F. & Suzuki, K. *Nature* **305**, 713 (1983).

100 years ago

PROF. J.P. Licherdopol writes from Bucharest, Roumania, that on January 1, at 6.13 a.m., two horizontal shocks of earthquake, from north to south and *vice versa*, were felt there, and were preceded by a loud noise, as of a distant train coming from the north. The furniture was slightly shaken and crackings were heard. The atmosphere was calm, but charged with a very thick and persistent fog.

A series of ornithological observatories has been established throughout Austria-Hungary at the instance of Crown Prince Rudolf, with a view of paying special attention to the migrations of birds, as well as to their breeding habits. The work done by these stations is satisfactory enough; yet it has been found that a complete insight into the periodical movement of birds cannot be obtained so long as similar stations are not spread over the whole globe. The subject is to form one of the principal topics for discussion at the approaching Ornithological Congress, which will be held under the auspices of the Crown Prince at Vienna on April 16 next and the following days.

From *Nature* **29**, 244; January 10th, 1884.

Medical genetics

Molecular diagnostic medicine

from Peter Newmark

FOR too many years those who felt obliged to respond to the taunt that molecular biology, for all the medical research money it had soaked up, had never done anything to help diagnostic medicine, could, at best, just about fall back on the case of thalassaemias. The extent to which the situation has changed in the last few years was well illustrated at a recent Titisee conference* and has since been spectacularly demonstrated in the case of Huntington's disease.

A key to much of the recent progress, including that with Huntington's disease, has been the use of restriction fragment length polymorphisms as genetic markers of disease. Remarkably, they provide a means of detecting the presence of a hereditary genetic lesion, even when the lesion itself is completely elusive. The theoretical basis of that approach was described in great detail three years ago (Botstein, D., White, R.L., Scolnick, M. & Davis, R.W. *Am. J. hum. Genet.* 32, 314; 1980).

Restriction fragment length polymorphisms are the fortuitous result of variations in DNA sequence which are detectable because they generate (or eradicate) cutting sites (short, specific sequences of base pairs) for one or another restriction enzyme. Therefore the restriction fragments produced by the enzyme from the two alleles will differ in length. Since fragments of different length can be separated (by agarose gel electrophoresis) and detected with specific DNA probes (Southern hybridization) it is possible to determine (prenatally, if necessary) which form of a polymorphic sequence is carried by any individual and through any family.

To make use of that information for diagnosis it is necessary to find at least one and preferably several polymorphisms that are close enough to the chromosomal locus of the disease to minimize the chances of becoming separated from it by genetic recombination during formation of gametes.

It is important to remember that the polymorphisms are not the result of the lesion that caused the disease but simply a pre-existing variation in the population at large. Therefore which of the polymorphic restriction fragment lengths will be linked to the disease in any one family will depend upon which one existed on the ancestral allele in which the lesion arose and from whose bearer the family is descended: the case of Huntington's disease illustrates how two families have a different restriction fragment marker linked to the

disease. Moreover, there is always a chance, sometimes high, that the same restriction fragment that characterizes the ancestral lesioned allele will be 'imported' into the family on the same chromosome as an unlesioned allele — hence the necessity for family studies.

In Titisee, Duchenne muscular dystrophy and the Fragile X mental retardation syndrome were discussed as examples of diseases for which restriction fragment length polymorphisms should aid both prenatal diagnosis and, eventually, the ability to identify the mutant gene. Both diseases are known to be located on the X chromosome and so it has been immediately possible to narrow down the hunt for restriction fragment length polymorphisms that lie close enough to the mutant gene usually to remain attached to it. K. Davies (St. Mary's Hospital Medical School, London) briefly described a polymorphism that is loosely linked to the 'fragile site' near the tip of the X chromosome whose breakage is associated with mental retardation; a more closely linked polymorphism is one that is known by its association with the gene for the blood coagulation factor IX (G. Camerino *et al. Nature* 306, 761; 1983).

For Duchenne muscular dystrophy, Davies offered two polymorphisms, one on either side of the mutant gene. Neither is linked closely enough for confident prenatal diagnosis in affected families but they have nonetheless proved useful for carrier detection, particularly in families where both occur. Unfortunately, between one-third and one-half of muscular dystrophy cases are the result of new mutations and so will never be detectable without prenatal population screening, which is unthinkable.

The distance of each polymorphism from the mutant gene — around 15×10^6 base pairs — will also make difficult the other goal, that of identifying the mutant gene by working in from the polymorphisms. Davies makes the reasonable assumption that the mutation is in a gene that is normally expressed and therefore should be identifiable by an RNA transcript that corresponds to the gene. She estimates that the search is for one transcript in about a hundred that will be specified by the sequences lying between the two polymorphisms.

An alternative approach was described by E. Southern (MRC Mammalian Genome Unit, Edinburgh). It begins with some very rare female sufferers from muscular dystrophy (it being a recessive disease, females are usually only carriers). The female sufferers are characterized by the presence of an X chromosome trans-

location, always involving the same band of the X chromosome but with different autosomes partaking in the reciprocal cross. Southern's assumption is that the X chromosome breaks at the muscular dystrophy gene on the active member of the pair of X chromosomes, and that the sequence across the breakpoint should therefore lead directly to the relevant gene. (If the gene is not at the breakpoint it must be close enough to it to be inactivated as a consequence of the translocation.) To help overcome the difficulties of the direct approach, his group is also trying to produce a probe for human ribosomal genes that should help them in a specific case where it is the ribosomal genes from chromosome 21 that abut the broken end of the X chromosome as a result of the reciprocal translocation.

Lesh-Nyan is also an X-linked recessive disease but one in which the genetic lesion is known. It is the result of a deficiency of hypoxanthine-guanine phosphoribosyl transferase. However, according to C.T. Caskey (Baylor School of Medicine, Houston), there is seldom a lack of messenger RNA, presumably because the genetic lesion is usually a point mutation. Defects at the level of DNA have been detected in about half of the 28 families investigated but in no two cases are they the same. The optimal means of prenatal diagnosis of Lesh-Nyan disease is not yet certain but the use of restriction fragment

THE International Titisee Conferences, held in Titisee in the Black Forest, have always been sponsored by the German company of Boehringer Ingelheim. Sponsorship used to come through their subsidiary, Dr Karl Thomae GmbH, but for the past year the new Boehringer Ingelheim Foundation, under its director Dr Hasso Schroeder, has taken over the job.

In addition to continuing to hold two or three Titisee conferences each year, the foundation has announced two other ways in which it will support basic research in medical sciences in the Federal Republic of Germany. First, it will sponsor an annual scientific lecture series in which the lecturer not only speaks at several academic centres but also spends a day or two at each as a consultant. Second, it will provide three types of grant. There are to be grants of one to three years for postgraduates working on basic clinical research projects; grants to industrial researchers, not necessarily in the pharmaceutical industry; and grants to cover trips of not more than sixty days to learn a technique or attend a conference.

The foundation has a budget of about DM 1.5 million (£0.4 million) this year and expects to have half as much again next year. Decisions are made by a board of trustees. □

*The International Titisee Conference of the Boehringer Ingelheim Foundations on Impact of Modern Molecular Genetics on Human Biology and Medicine, was held on October 5-9, 1983.

length polymorphisms may prove to be best. Already this seems to be the case for phenylketonuria in which there may also be no consistent or readily detectable lesion accounting for the deficiency of phenylalanine hydroxylase (S.L.C. Woo *et al.* *Nature* 306, 151; 1983).

Another, very elegant, use of restriction fragment length polymorphisms (R. White, University of Utah School of Medicine, Salt Lake City) showed that retinoblastoma is the result of development by at least two different mechanisms, of homozygosity for the mutant allele on chromosome 13 (see *News and Views* 305, 761 and Cavanee *et al.* *Nature* 305, 779).

It was left to D. Weatherall (John Radcliffe Hospital, Oxford) to address some of the practicalities of prenatal detection of genetic diseases based on the accumulated evidence of thalassaemias. For example, will the new technique of sampling chorionic villi in pursuit of first trimester diagnosis have an acceptable risk

rate? It is too soon to know, said Weatherall. Why is clinical phenotype not always similar for one class of genetic lesion, making genetic counselling that much harder? Co-existent α -thalassaemia can sometimes dampen down the effects of homozygous β -thalassaemia, explained Weatherall, by way of example. Will prenatal population screening for genetic lesions ever be justified? Very rarely. But in the case of β -thalassaemia in Cyprus where 20–30 per cent of the population are carriers, 1–2 per cent of the children are homozygous and will die before reaching adulthood. In 95 per cent of the homozygous children the β -globin mutation is directly detectable and the co-existence of β -thalassaemia that reduces the risk of death from the disease can also be diagnosed. Here, there is a good case for prenatal screening of the population. □

Peter Newmark is Deputy Editor of Nature.

Space plasma physics

Antarctic research by satellite link

from Dyfrig Jones

WHAT do long term experiments in Antarctica have in common with experiments on scientific satellites? One obvious answer is their remoteness. And in what way do they differ? Data from satellites are available in real time and can even be analysed directly by dedicated computers on the ground, allowing scientists, or even the computers, rapidly to change how the experiment is operating. But data from experiments in Antarctica are usually stored on magnetic tape and collected once a year by ship — so producing a long delay between data acquisition and analysis, and an even greater delay before the experiment can be reconfigured. The delay is particularly frustrating in the increasing number of studies where satellite and Antarctic data are correlated in an effort to understand highly complex magnetospheric processes, because the spacecraft data have to be shelved until the Antarctic data arrive. Now, steps are being taken at the British Antarctic Survey, to overcome the problem, by transmitting scientific data from Antarctica to Cambridge via an INMARSAT geostationary communications satellite.

The space plasma physics section of the British Antarctic Survey (BAS) with the physics department, University of Sheffield, has operated VLF radio receiving equipment at Halley Station, Antarctica for a number of years. The main thrust of the research lies in the analysis of natural and man-made radio waves in the frequency range up to 10 kHz, the aim being to understand the generation and propagation of the natural waves and to

determine what influence man-made waves may have on the magnetosphere. NASA's satellite Dynamics Explorer 1 is in an orbit admirably suited for correlative measurements with Halley, and a close collaboration exists between BAS and the Universities of Stanford and Iowa, and NASA. It greatly helps the project if the Antarctic data are readily available.

A satellite terminal was installed at Halley in January 1983. The first VLF data were transmitted to Cambridge in July. VLF wave experiments are highly demanding in terms of the amount of information that has to be acquired and telemetered. This is because natural waves, which occur randomly, may vary rapidly in frequency with time. To ensure that most significant events are captured, a wide band, typically 10 kHz, needs to be monitored continuously. Further, studies often require that the waves be recorded on more than one sensor. At Halley, two orthogonal vertical loop antennae are used, the minimum required for obtaining information on the waves' direction of arrival. If signals with a 10-kHz bandwidth are sent in digital form via satellite, 350,000 bits of information need to be transmitted for each second of data. For a bit-rate of 2.4 kbits s⁻¹, each second of data takes 2.5 minutes to transmit. Obviously, there is a strong financial incentive to decrease transmission time, and investigations are under way to see how this can be done without losing scientifically significant data.

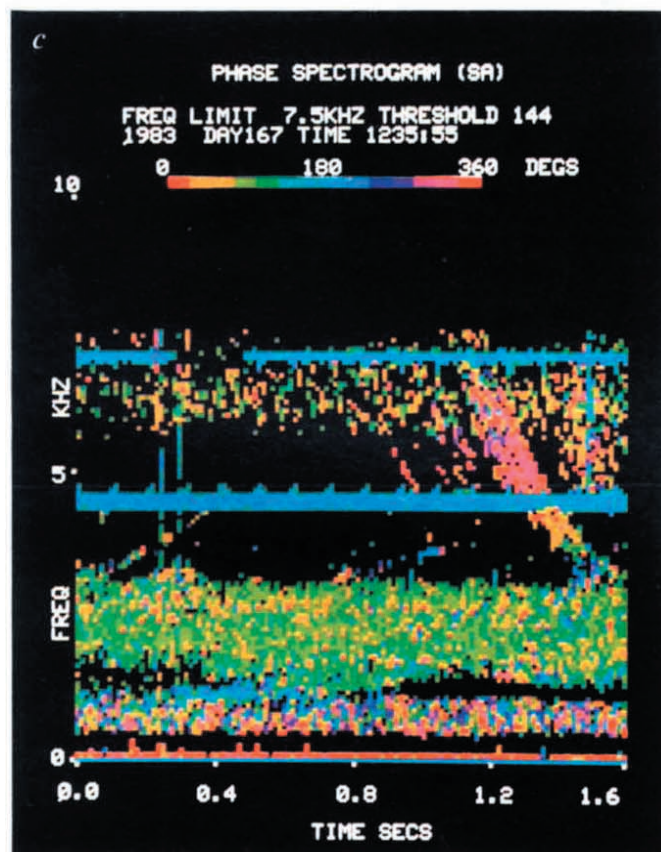
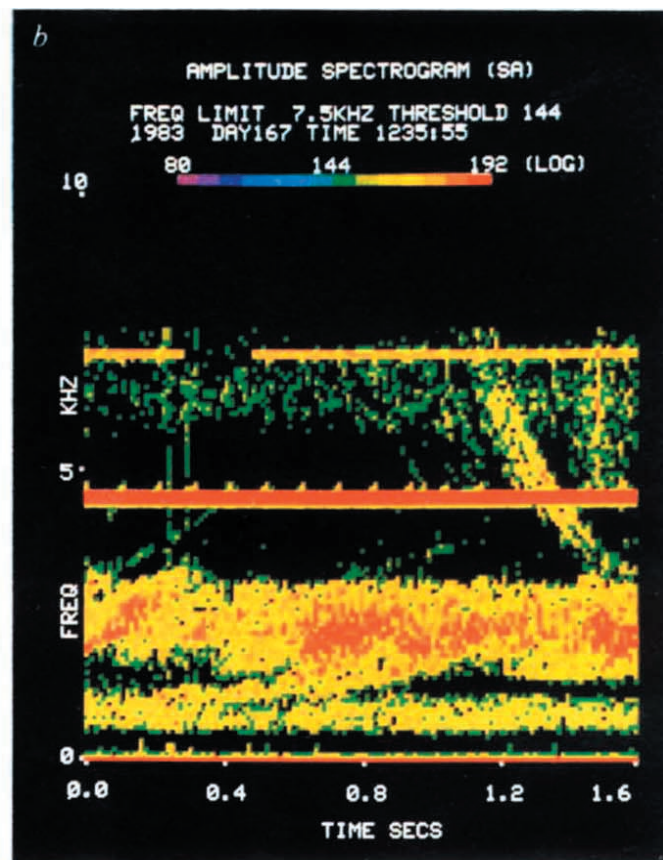
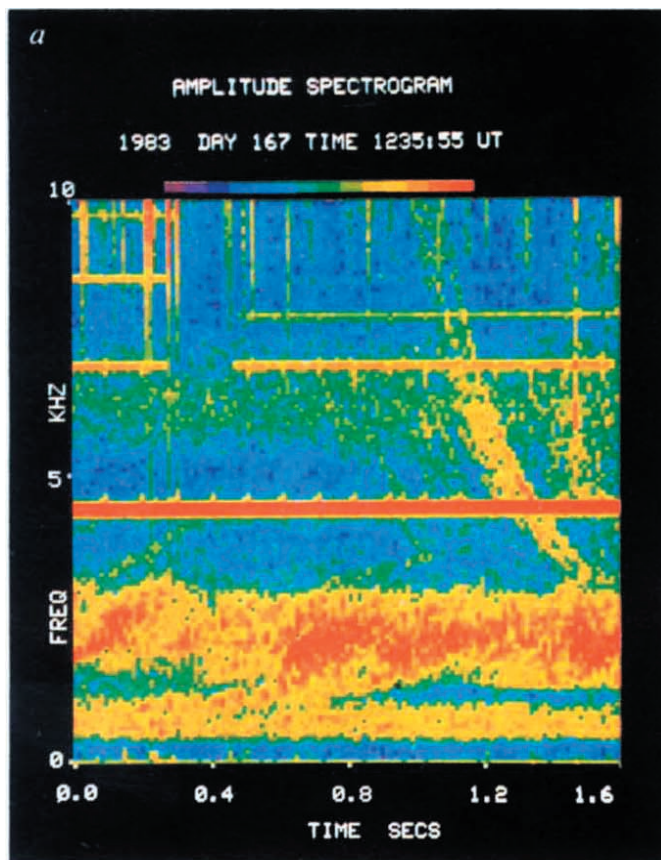
On the ESA satellite GEOS, which carried a sophisticated wave experiment, a similar problem was resolved by incorporating a digital correlator on the spacecraft.

This first computer in space calculated the auto- and cross co-variance functions of signals which, after reception on the ground, were Fourier transformed to produce dynamic spectra. The technique achieved a data compression factor of 20; this could have been increased with more powerful on-board processing. One technique being investigated using GEOS data by BAS, collaborating with the Laboratoire de Physique et Chimie de l'Environnement, Orleans, is the extraction of signals using cross-spectral phase information. The method is now being applied to Halley data, the preliminary results being shown in the figure. Signals from the orthogonal loops were digitized at Halley and despatched, without pre-processing, to Cambridge via satellite. Cross-spectral analysis was performed on the Cambridge IBM 3081 computer, the resulting spectrogram being shown in *a*. The constant frequency line near 5 kHz propagated from a VLF transmitter at the US station Siple. Intermittent lines at higher frequencies are due to navigation transmitters. The signals sweeping down from 10 kHz to 3 kHz are whistlers, that is, radio waves from lightning in the Northern Hemisphere which have propagated along the magnetic field line to Halley. The analysis of these gives invaluable information on plasma conditions out to 30,000 km from Earth.

The emissions below 3 kHz are natural waves produced in the Earth's magnetosphere. All the blue area is background noise. To remove this, the same data were pre-processed at Halley and only the spectral amplitude above a certain threshold was transmitted via satellite. The result is shown in *b*. Virtually all the signals visible in *a* are retained but the noise has been rejected; the maximum frequency was limited to 7.5 kHz. Figure *c* shows the corresponding cross-spectral phase spectrogram, the phase between signals on the orthogonal antennae being colour-coded. The relative phase of the waves from 1 to 3 kHz is as expected for a right-hand polarized wave propagating down from the ionosphere above the receiving site. The phases of the whistlers and the emissions below 1 kHz are different, implying that they have propagated to the receiver from a considerable distance in the Earth-ionosphere waveguide. Further computations will determine their direction of arrival.

These examples serve to illustrate not only the potential of satellite links for scientific research at remote stations, but also the importance of data pre-processing if the link is to be efficiently utilized. It should be added that the satellite link provides two-way communications and it is envisaged that experiments at Halley will be reprogrammed from Cambridge in much the same way as a satellite experiment is reconfigured by ground command.

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a, Digital cross-spectrogram of VLF signals received on orthogonal loop aerials at Halley, Antarctica at 12.35 UT on June 16, 1983, just before mid-winter. Spectral amplitude is colour-coded according to the colour bar shown above the spectrogram, red representing the most intense waves. The signals were digitized at Halley, transmitted via communications satellite to the UK, and spectrum analysed by computer at Cambridge. Without a satellite link these data would not have been available until March–April, 1984.

b, This is in the same format as *a* but the spectrum analysis had been performed at Halley and only spectral amplitudes above a certain threshold and in a restricted frequency range (0–7.5 kHz) were transmitted via satellite. This reduced the information sent as compared to that in *a* by more than 60 per cent but the scientifically useful signals were retained.

c, The cross-spectral phase spectrogram corresponding to *b*. The relative phase between signals received on the orthogonal aerials at Halley is colour coded as indicated by the colour bar above the spectrogram. Red and blue imply linear polarization, as expected for waves which have propagated over relatively large distances in the earth-ionosphere waveguide. Green represents right-hand elliptical or circular polarization corresponding to whistler-mode waves propagating down from the ionosphere above the receiving site.

Particle physics and cosmology

New light on heavy light

from John D. Barrow and R. R. Burman

DURING the past three years it has been extremely fashionable to consider the results of endowing the neutrino with a non-zero rest mass, and there now exists experimental evidence to suggest that this idea may one day pass from the realm of the fashionable to that of the factual¹. However, while a small neutrino mass has dramatic consequences for astrophysics² it has a surprisingly small effect upon the structure of microphysical theories. This is because the neutrino does not mediate the weak interaction and hence the reciprocal of its mass does not determine the range of the weak force. If we choose to endow the photon with a non-zero mass the consequences will be more far-reaching: the masslessness of the photon is responsible for the infinite range and gauge invariance of the electromagnetic interaction.

Classical electrodynamics with a non-zero rest mass is described by the Proca equations^{3,4} which reduce to Maxwell's when the photon mass (m_γ) is zero. These equations contain a characteristic length scale, $\lambda = \hbar/cm_\gamma$, and a small non-zero m_γ will lead to significant physical effects on the scale of the photon Compton wavelength λ . The length λ is an effective 'range' of the electromagnetic interaction here and static electric and magnetic fields will exhibit an exponential, $\exp(-r/\lambda)$, fall-off at distances $r > \lambda$ from sources. Other new features include a systematic variation of the velocity of light with frequency and a violation of Amperes law.

In an intriguing letter to *Nature*, Georgi, Ginsparg and Glashow⁵ have resurrected the idea of a non-zero photon mass, but with a new twist. They suggest that in addition to the usual low energy symmetries $SU(3) \times SU(2) \times U(1)$ governing the strong, weak and electromagnetic interactions respectively, there exists an additional $U'(1)$ symmetry in nature, and oscillations occur between the different 'photons' which mediate the $U(1)$ and $U'(1)$ interactions. This structure allows the 'ordinary' massless photon to obtain a mass, (see also refs. 6 and 7). The consequent classical electrodynamics would obey Proca rather than Maxwell field equations. This photon oscillation picture provides a natural scheme wherein a photon mass can enter, and is entirely analogous to the more familiar idea of neutrino oscillations. Georgi *et al.* point out some observational consequences that arise even if m_γ is so small as $\sim 5 \cdot 10^{-18}$ eV/c². The oscillation of photons between cosmological relic distributions of the two kinds at 2.9 K (the measured temperature of the cosmic microwave background radiation) leads to small distortions from the precise Planckian spectral form that would arise if m_γ

were zero. These distortions happen to be in the same sense and of the same magnitude as the purported spectral distortions measured by Woody and Richards⁸ balloon-borne experiment in 1979. It is only fair to say that cosmologists have been wary of drawing any conclusions from the existence of these distortions because of their ambiguous statistical significance and the real possibility of systematic error. More exciting is the prediction of Georgi *et al.* that a definite sinusoidal variation of flux with wavelength should be observed shortward of 2 cm if the above spectral distortions arise from photon oscillations.

The photon mass level of $5 \cdot 10^{-18}$ eV/c² required for measurable effects to exist is an interesting one. It is consistent with the observational^{4,9} limits on m_γ obtained by direct experiments and all other limits obtained until the early 1970s which require only that $m_\gamma \leq 6 \cdot 10^{-16}$ eV/c². However, there exist a variety of astrophysical limits on m_γ , which Georgi *et al.* do not consider, that appear to rule out a value so high as $5 \cdot 10^{-18}$ eV/c² by a very wide margin. They all arise from detailed consideration of astrophysical objects in which magnetic fields, and hence the Maxwellian form of

electrodynamics, play a key role in maintaining equilibrium or creating long-lived stable structures. Following a suggestion of Goldhaber and Nieto⁴, Byrne and Burman⁹ derived an upper limit of $\sim 3 \cdot 10^{-20}$ eV/c² on m_γ by considering the electric current density and concomitant Joule heating in the interstellar medium. Barnes and Scargle¹⁰ produced a similar limit from a study of what appear to be magneto-acoustic waves ('wisps') in the Crab Nebula. The existence of moderately dense, magnetized ($1\text{--}2 \cdot 10^{-5}$ gauss) interstellar gas clouds which appear to have contracted across the Galactic magnetic field, compressing flux, leads to an upper limit on m_γ of about 10^{-25} eV/c² in order that the repulsive Proca stresses do not overwhelm the effects of gravity and prevent collapse^{9,11}. A similar physical effect would prevent the equilibrium of interstellar gas in the disc of our Galaxy unless $m_\gamma \leq 10^{-25}$ eV/c². Finally, it has been argued by Chibisov¹² that the magnetically-dominated interstellar plasma observed in the Small Magellanic Cloud would not exist in magneto-gravitational equilibrium unless the photon Compton wavelength, λ , which sets the effective range of the interaction, exceeds the characteristic field scale ~ 3 Kpc. This leads to a very restrictive upper limit on m_γ of about 10^{-27} eV/c². All these limits should be regarded as uncertain by about an order of magnitude numerically and each has associated experimental and theoretical uncertainties. We have

Table 1 Summary of limits on m_γ

m_γ limit	Site	Uncertainties
10^{-10} eV/c ²	Variation of light speed with frequency (terrestrial) ^{4,19}	Irregularity of medium in which light propagates; possible measurement error
5×10^{-12}	Dispersion of radiation from Crab pulsar ^{4,26}	Dispersion in interstellar plasma mimics the effect of photon mass
10^{-14}	Deviations from Coulomb's law (laboratory) ^{4,18}	
3×10^{-14}	Terrestrial Schumann resonances ²⁰	Cavity geometry
2×10^{-15}	Geomagnetic field variation on and near Earth ^{4,21,22}	Regularity of field; incomplete mapping of field; externally generated fields
4×10^{-16}	Pioneer-10 measurement of Jovian magnetic field ²³	Regularity of field; perturbation by external fields
2×10^{-16}	No low-frequency cut-off for hydromagnetic waves in the Earth's magnetosphere and solar wind ²⁴	Limited data; interpretation of a positive results could be non-unique
2×10^{-20}	Magneto-acoustic 'wisps' in Crab Nebula ¹⁰	'Wisps' may not be magneto-acoustic waves
2×10^{-20}	Galactic magnetic field ^{4,9}	Properties and uniformity of interstellar medium
10^{-25}	Interstellar gas clouds ^{4,9}	Initial conditions in plasma that forms the gas clouds
10^{-25}	Interstellar gas in Galactic disc ^{4,9}	Regularity of field lines and plasma
10^{-27}	Interstellar gas in Small Magellanic Cloud ^{12,25}	Regularity of field lines and plasma; theory not presented in detail
10^{-33}	Smallest mass measurable in age of the Universe	

BOX 1: PROCA EQUATIONS

$$\nabla \times \mathbf{E} = -\frac{1}{c} \frac{d\mathbf{B}}{dt}, \quad \nabla \cdot \mathbf{B} = 0$$

$$\nabla \times \mathbf{B} = \frac{4\pi}{c} \mathbf{j} + \frac{1}{c} \frac{d\mathbf{E}}{dt} - \frac{m_\gamma^2 c^2}{\hbar^2} \mathbf{A}$$

$$\nabla \cdot \mathbf{E} = 4\pi\rho - \frac{m_\gamma^2 c^2}{\hbar^2} \phi$$

$\mathbf{A}$ and ϕ are the space-time components of the 4-vector potential A_μ , $\mathbf{E}$ and $\mathbf{B}$ the electric and magnetic 3-vectors; $\mathbf{j}$ the current density; ρ the charge density. The equations are not gauge invariant if $m_\gamma = 0$, and the Lorentz gauge ($\nabla \cdot \mathbf{A} = 0$) must be employed for consistency.

mentioned some of these uncertainties in a tabular summary of the experimental situation. It remains to be seen whether the huge superficial conflict between the astrophysical upper limits on m_γ and the value of about 5.10^{-18} eV/ c^2 required for observable effects on the 2.9 K background can be side-stepped by a more rigorous scrutiny of the data and a wider examination of the theoretical alternatives.

One of the most interesting consequences of photon oscillations would be, as Georgi *et al.* remark, the striking fact that observations of what might be the lowest energy scale in nature, $m_\gamma \leq 10^{-18}$ eV/ c^2 , would tell us definite things about physics at ultra-high energies $\sim 10^{15}$ – 10^{19} GeV/ c^2 . In the last decade the most fruitful approach to the problem of elementary particles has been to follow the unified gauge theory idea to its logical and most elegant conclusion, namely the existence of a 'grand unification' of the $SU(3) \times SU(2) \times U(1)$ symmetries of the strong and electro-weak interactions within a simple gauge group like $SU(5)$, as first suggested by Georgi and Glashow¹³ in 1974. However, if there exists a non-zero photon mass and an $SU(3) \times SU(2) \times U(1) \times U'(1)$ symmetry at low energy, then it is not possible for this low-energy behaviour to have resulted from the breaking of a single grand unified symmetry, like $SU(5)$, at high energy. A proof of this result first appeared in a paper by Calogheracos, Dombey and Kennedy¹⁴ and its truth is realized independently by Georgi *et al.* in their *Nature* letter. This theorem comes about on account of a fundamental difference in behaviour between a theory such as quantum electrodynamics (QED), which is invariant under the Abelian gauge group $U(1)$ (the group under multiplication by complex numbers of unit modulus), and a theory such as quantum chromodynamics (QCD), which has a non-Abelian gauge group ($SU(3)$ in the case of QCD).

Many years ago Stueckelberg¹⁵ showed for QED that, even if the $U(1)$ gauge invariance is broken by the addition of a small photon mass, the theory still exists and the limit $m_\gamma \rightarrow 0$ is smooth. Van Dam and Veltman¹⁶, however, showed in 1970 that this was not the case for Yang-Mills theory (or $SU(2)$), which is the simplest non-Abelian gauge theory. Here, if the

gauge particle is given a mass m , the limit $m \rightarrow 0$ does not produce the original massless theory (it is, in effect, a 'singular perturbation' of the massless theory). Dombey and Vayonakis¹⁷ then showed that this peculiar behaviour arose from the existence of a longitudinal polarization state when the gauge particle possesses mass. In an Abelian theory gauge invariance causes this longitudinal mode to decouple when $m_\gamma \rightarrow 0$ (as it does in classical electrodynamics), whereas in a non-Abelian theory the gauge invariance is more complicated and, as a result, the longitudinal mode fails to vanish in the zero-mass limit.

In a hybrid theory, such as electro-weak $SU(2) \times U(1)$ or the $SU(3) \times SU(2) \times U(1) \times U'(1)$ proposed by Georgi *et al.*, the QED situation persists (because of the $U(1)$ subgroup) and there can be an arbitrarily small photon mass. On the other hand, if electrodynamics were contained in a Grand Unified Theory such as $SU(5)$ which, being a simple group, has no $U(1)$ subgroup, then there would be no way of adding a small photon mass term within normal theories without violating charge conservation.

The discovery, therefore, of evidence for photon oscillations in the way suggested by Georgi *et al.* would be dramatic: not only would it show Grand Unification to be a false route towards understanding elementary particles but it would also compromise physicists' central article of faith — gauge invariance — as the most reliable guide to nature's ultimate structure.

Finally, it is worth pondering that a photon mass could well exist at a level below the reach of all direct experimental techniques, however accurate. The Heisenberg Uncertainty Principle gives the lowest mass m that can be measured in the

age of the universe $T \sim 5.10^{17}$ sec as $m \sim \hbar T^{-1} c^{-2} \sim 10^{-33}$ eV/ c^2 — surprisingly close to the strongest astrophysical limit on m_γ . Photons with a mass less than 10^{-33} eV would have a Compton wavelength greater than the size of the observable Universe, $cT \sim 1.5.10^{28}$ cm. Such incoherent quantum wave functions would, at the very least, shed new light on determinism. $\square$

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1. Lyubimov, V. A., *et al.* *Sov. Phys. JETP* **54**, 616 (1980).
2. Barrow, J. D., *Fund. Cosmic Phys.* **8**, 83 (1983).
3. Proca, A. *J. Phys. Radium Ser. VII* **8**, 23 (1936).
4. Goldhaber, A. S. & Nieto, M. M. *Rev. Mod. Phys.* **43**, 277 (1983).
5. Georgi, H., Ginsparg, P. & Glashow, S. L. *Nature* **305**, 765 (1983).
6. Okun, L. B. *Sov. Phys. JETP* **56**, 502 (1982).
7. Barr, S. M., Reiss, D. B. & Zee, A. *Phys. Rev. Lett.* **50**, 317 (1983).
8. Woody, D. P. & Richards, P. L. *Phys. Rev. Lett.* **42**, 925 (1979).
9. Byrne, J. C. *Astrophys. Sp. Sci.* **46**, 115 (1977).
10. Barnes, A. & Scargle, J. D. *Phys. Rev. Lett.* **35**, 117 (1975).
11. Murphy, G. L. & Burman, R. R. *Astrophys. Sp. Sci.* **56**, 363 (1978).
12. Chibisov, G. V. *Sov. Phys. Usp.* **19**, 624 (1976).
13. Georgi, H. & Glashow, S. L. *Phys. Rev. Lett.* **33**, 451 (1974).
14. Calogheracos, A., Dombey, N. & Kennedy, A. D. *Phys. Lett.* **104B**, 444 (1981).
15. Stueckelberg, E. C. G. *Helv. Phys. Acta* **14**, 51 (1941).
16. van Dam, H. & Veltman, M. *Nucl. Phys. B22*, 397 (1970).
17. Dombey, N. & Vayonakis, J. *Phys. A* **9**, 1381 (1976).
18. Williams, E. R., Faller, J. E. & Hill, H., *Phys. Rev. Lett.* **26**, 721 (1971).
19. Al'pert, Y. L., Migulin, V. V. & Ryazin, P. A., *ZH. Tech. Fiz.* **11**, 29 (1941).
20. Jackson, J. D. *Classical Electrodynamics* (Wiley, New York, 1962).
21. Schrödinger, E. *Proc. Roy. Irish Acad.* **A49**, 135 (1943).
22. Frank, L. A. *J. geophys. Res.* **72**, 3753 (1967).
23. Davis, L., Goldhaber, A. S. & Nieto, M. M. *Phys. Rev. Lett.* **35**, 1402 (1975).
24. Gintsburg, M. A. *Sov. Astron.* **7**, 536 (1964).
25. Dolgov, A. D. & Zeldovich, Y. B. *Rev. Mod. Phys.* **53**, 1 (1981).
26. Feinberg, G. *Science* **166**, 879 (1969).

Astronomy

Astronomical angular accuracy

from David W. Hughes

PRACTICAL astronomy before the middle of the nineteenth century was mainly concerned with the measurement and cataloguing of the positions of stars and planets. Large-radius sextants, quadrants and circles were used. Accuracy was paramount, and depended on three main factors: accurate graduation of scales, careful alignment and skilful technique.

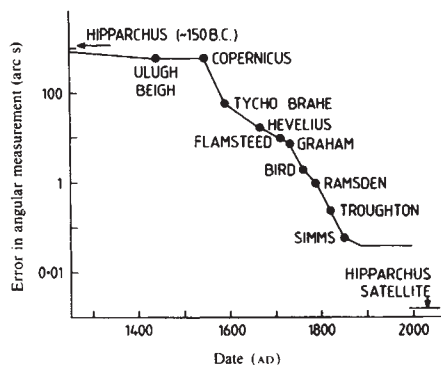
In many cases the quest for accuracy was fuelled by the demands of the theoretician. A typical example concerns stellar parallax. The Copernican insistence that the Earth orbited the Sun had the concomitant consequence that close-by stars should execute annual displacements as seen against the more distant 'fixed' stars. Proxima Centauri moves about 1.5 arc s because of this process. Three centuries elapsed between the prediction of this effect and its observation, when the craftsmen and astronomers and their

instruments and techniques caught up in the 1830s.

The advance in angular accuracy is summarized in the figure, and the subject has been reviewed by Allan Chapman of Wadham College, Oxford in a recent edition of the *Journal for the History of Astronomy* (**14**, 133; 1983).

Three important developments in instrument-making were responsible for the changes shown in the figure. The first was introduced by Tycho Brahe. He augmented the normal radial scale divisions on the limbs of instruments by interspersing a series of diagonal lines. This enabled him to read the scale to a fraction of a degree whilst still retaining the small compact stable features of the instrument. Brahe also improved the sights of sextants and quadrants, thus eliminating most of the alignment errors.

Before 1660 angular measurement was



The observational error in the astronomical measurement of stellar and planetary positions plotted logarithmically against the year. The data have come from H. Mineur (see H.T. Pledge, *Science since 1500*, HMSO; 1939), A. Chapman (*J. hist. Astr.* 14, 133; 1983) and European Space Agency DP/PS(78)13.

also limited by the 1-arc-min resolution power of the human eye. The adoption of the telescopic sight and the micrometer overcame this barrier and the angular error fell to 15 arc s by 1700 and 8 arc s by 1725. This made it possible to detect stellar aberration (small positional wanderings due to the vectorial addition of the velocity of light to the Earth's orbital velocity) and nutation (an 18.6-year wobble in the Earth's spin axis produced by the gravitational influence of the Moon and

planets).

The third breakthrough came in the late eighteenth century. Instrument-makers Jesse Ramsden and Edward Troughton replaced the quadrant with the full circle. Circles could be rotated thus enabling the graduations to be cross-checked with micrometer microscopes. By 1800 the error had been reduced to 0.5 arc s. 1850 saw the installation of G.B. Airy's transit circle at Greenwich. Troughton and Simms were responsible for the optics and instrumentation. Six cross-checking micrometer-microscopes placed at 60° intervals around the circle gave a final accuracy of 0.06 arc s.

Unfortunately the accuracy of astronomical measurements of stellar position has only improved very slightly since then. The accuracy of the main applications of astrometry — the motions of the Moon and planets, the kinematics and dynamics of the galaxy, stellar mass determination and the rotation of the Earth and polar wandering — have also remained almost stationary. The next leap forwards will come from the ESA Hipparcus satellite. This is designed to measure stellar position to an accuracy of ± 0.0015 arcs and stellar parallax to ± 0.002 arcs. □

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Archaeology

Setting Stonehenge straight

from Christopher Chippindale

FOR years the dismal state of Stonehenge, Europe's premier prehistoric monument, has been a disgrace — and an embarrassment to British archaeology. Always the most famous English ancient monument, it has become even more popular since archaeo-astronomers tried to identify it as a neolithic observatory. Yet many of its visitors, up to 800,000 annually, and two-thirds of them from abroad, find the place a sad let-down — hemmed in by busy main roads, approached through a nasty grey concrete tunnel, and each June the unwilling focus of an immense drug and rock music festival.

Partly it is a simple question of numbers. Stonehenge is rather a small monument — the central ruin with its three standing trilithons is only thirty-five paces across — and the grass there cannot survive the tramp of a million feet. Since 1978 the Department of the Environment (DoE), the government agency responsible, has closed the centre. Instead visitors are shepherded along a tarmac path, camouflaged a chemical shade of green, well away from the stones. In consequence it is impossible properly to see those features, the mortise-and-tenon joints of the lintels, the blue-stone settings, the solar alignment of the

axis, that make Stonehenge extraordinary and unique. And since aids for visitors are minimal, it is hard to know just what you should be looking out for.

The romantic ideal is to see Stonehenge alone, preferably by moonlight and with a storm brewing (it is still strongly recommended but is now very hard to manage). Over 200 years ago, William Stukeley, the first great student of Stonehenge, grumbled about its 'infinite number of daily visitants'. It was a famous shambles at Victorian bank holidays, with 'vanloads of uproarious humanity, dressed in all the colours of the rainbow, and in many others of aniline origin', and the dominant sound not lark-song but the crash of souvenir-hunters' hammers on the stones¹.

So great was the threat, during the 1920s, of a suburban sprawl of shapeless development — bungalows, cafés, an intensive pig-farm — crowding in on Stonehenge, that a public appeal bought 700 hectares of surrounding land and gave it to the National Trust, which would preserve Stonehenge for ever in a sweep of empty chalk downland. There were schemes to suppress the main A344 road which ran a couple of metres from the Heel Stone, so that the "circle itself seems almost submerged by the congestion,

vulgarity, speed, and noise". But the road stayed open and soon spawned a car-park hard by the stones. (Calls for lavatories were at first resisted: "after all, the whole Plain is available for the convenience of the public".) Over the years, more facilities have grown on that site — extra space for cars several times, lavatories (both underground and up in the air), a café, souvenir shop, and bunker for the DoE's guardians — as well as a tunnel access-way to save the dangerous crossing of the road².

After four decades of such piecemeal growth, Stonehenge presents a classic case of how a cultural resource should not be managed. The main road across its edge is still open. The facilities are too close and too obtrusive, but because the site is cramped they are also inadequate. Management is bureaucratic and remotely directed from London, while the DoE shows no sign of imagination or initiative, or even of noticing the enormous advances being made elsewhere in managing and displaying historic monuments. It has suppressed the report of an independent working party³ which recommended new facilities at a sensible distance and on a sensible scale. Instead it wants to enlarge on the present site, which is on the land bought by public subscription expressly to prevent tasteless development. The National Trust, recently bruised by a very public row over letting the air force develop a military bunker on another of its holdings, is, with every reason, declining to cooperate. And since the National Trust is the actual landowner, it has an effective veto.

In April 1984, the DoE will lose control over Stonehenge, which will pass to a new, autonomous Historic Buildings and Monuments Commission. Certainly, the new commission will know what a disaster it inherits — one of its commissioners calls Stonehenge today a 'squalid scandal' — and there is every hope it will abandon the fossil attitudes of the old regime.

The downs around Stonehenge contain, within a few square kilometres, an astonishing concentration of prehistoric sites⁴, currently the subject of remarkable field-work by Julian Richards of the Trust for Wessex Archaeology. That ancient landscape, with its barrows, henges, cursuses and boundary earthworks, is the environment into which its builders put Stonehenge. A new policy for Stonehenge gives the chance of an imaginative scheme to show the place in that context — to set it once again, as William Stukeley explained in 1740, "in clean and distinct areas, distant from profane buildings and traffic"⁵. □

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1. C. Chippindale, *Stonehenge Complete* (London 1983).
2. C. Chippindale, *Antiquity* 57, 172 (1983).
3. Stonehenge Working Party Report to the Department of the Environment (1979, unpublished).
4. RCHME, *The Environs of Stonehenge* (Edinburgh 1979).
5. W. Stukeley, *Stonehenge* (London, 1740).

Structure and evolution of the Himalaya–Tibet orogenic belt

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The 1981 French–Chinese expedition to Tibet focused on the Lhasa block, extending earlier coverage 400 km north of the Tsangpo suture. The Lhasa block stood between 10 and 15° N latitude over most of the Upper Cretaceous and Eocene and, if Gondwanian in origin, had detached from Gondwana by early Permian. Seismic profiles reveal a complex Moho topography resulting both from multiple continental thrusting and large-scale strike-slip faulting. Subduction related granitoids representing mixtures of mantle and crustal components and anatectic granitoids have been analysed and dated. This study emphasizes the role of smaller blocks in the accretion of the continental mosaic.

A 3-year programme of French–Chinese cooperation for the study of Tibet and the northern Himalayas began in 1980. Preliminary results of the first campaign have already been published^{1–4}, and a second campaign, started in 1981, has yielded much additional data and has clarified several significant problems. The 1981 results have been discussed at a French–Chinese conference⁵ and here we present a synthesis of the major results. Details of observations, laboratory measurements or modelling in each specialized field will be published separately. Some preliminary results from the 1982 campaign have been used when available.

We will summarize our results on a north–south cross-section, supposed to run from 28° to 32° N lat. not far from the longitude of Lhasa (Fig. 1). A geographical map of the area is shown in Fig. 2. Information gathered along trails which are perpendicular to the main trend of the section will be included whenever necessary. Observations start at the main central thrust (MCT) in the vicinity of Katmandu (Nepal) and extend northwards to the small town of Anduo, close to the administrative boundary between Xizang (Tibet) and Qinghai provinces.

Blocks and sutures

From a palaeogeographic standpoint, three blocks separated by two major suture zones have been recognized (Fig. 1). The Indus Tsangpo suture (ITS) marks the boundary between the Indian continent to the south and the Lhasa block to the north. The Lhasa block is in turn bounded to the north by the Bangong–Nujiang suture (BNS), with the Qangtang (or Tanggula) block lying to the north. On the basis of earlier work⁶, at least two other sutures, the Kokoxili (KS) and Chilian (CS) sutures, can be recognized farther north, before reaching the Tarim basin (Fig. 1). The four sutures will be labelled ITS, BNS, KS and CS with increasing age from south to north. The identification of three blocks and two sutures in southern Tibet has been proposed by Chang and Cheng⁶ and has been corroborated by further work, including French–Chinese work in 1980 and 1981^{1,2,5}. The key observations for such a distinction are strati-

graphical and are summarized in Fig. 1. We present here additional constraints provided by new data.

Earlier palaeomagnetic results for the Lhasa block come from the Middle to Upper Cretaceous Takena formation (red beds and Orbitolina limestones) and the Lingzizong volcanic formation of Palaeocene to Eocene age, in the vicinity of Lhasa^{4,7}. The authors concluded that the Lhasa block stood at a latitude of 20° N 90-Myr ago and at 10° N 65-Myr ago. Our results confirm only (and partly) the second of these results. During the 1981 mission, sampling has been extended north to Anduo. Laboratory measurements on 18 sites in Takena red beds revealed a multicomponent magnetization. A pre-folding stable component can be confidently isolated, with positive fold tests at a confidence level higher than 99%. Palaeomagnetic data do not indicate any significant latitudinal movement within the Lhasa block, suggesting that it formed a single unit before folding of the Takena formation (around 70 Myr ago?). Although it cannot as yet be demonstrated whether this magnetization is primary or a remagnetization acquired just before folding, the fact that all sites show a normal polarity suggests that magnetization was acquired before 80 Myr, the end of the Cretaceous Long Normal Interval. The corresponding palaeolatitude of Lhasa, possibly as late as 80 Myr, is 12° N. At the same time, the northern margin of India may have been between 20 and 30° S. Results from nine sites in the Palaeocene–Eocene Lingzizong formation indicate a palaeolatitude close to 15° N. Very few precise isotopic ages are available for the Lingzizong formation^{7,8}: they are a ⁴⁰Ar/³⁹Ar age of 60 Myr on an andesite and a K–Ar age of 48 Myr on an ignimbrite. Thus it appears that the Lhasa block remained approximately stationary in latitude for a period that extended at least from 80 to 60 Myr and may have been as long as from 100 to 48 Myr. Since the suturing of India to Asia, the block has moved about 20° north.

The most significant piece of new palaeontological information is given by fossil plant remains from the Takena formation. Immediately north of Lhasa, but also further north near Lake Peng-Co, close to Barda, new species of silicified woods

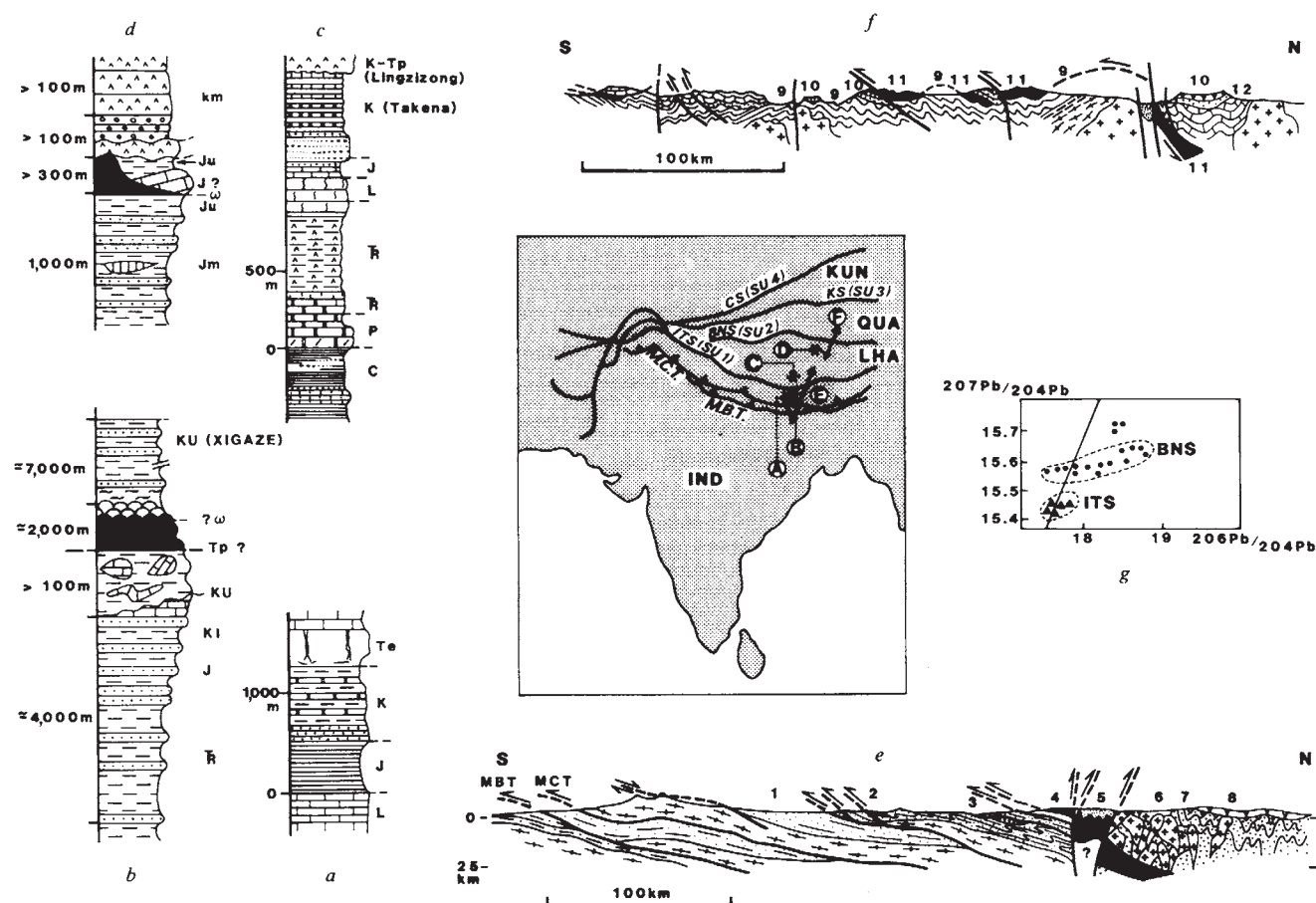


Fig. 1 Major tectonic blocks and boundaries in the Tibet-Himalaya. From south to north are the Indian (IND) plate, the Lhasa (LHA), Quantang (QUA) and Kunlun (KUN) blocks and the Asian (ASI) plate and the main boundary thrust (MBT), main central thrust (MCT) and Indus Tsangpo (ITS or SU1), Bangong Nujiang (BNS or SU2), Kokoxili (KS or SU3) and Chilian (CS or SU4) sutures. Stratigraphical sections from the southern (a) and northern (b) parts of South Tibet and from the southern (c) and northern (d) parts of the Lhasa block (see ref. 1 for details of sections a and c). e, Schematic cross-section through the ITS (see also ref. 37). 1, South Tethyan series (Indian shelf sediments); 2, North Tethyan series (Triassic and Jurassic of the Indian continental rise); 3, Upper Cretaceous or Palaeocene (?) wildflysh; 4, ophiolite series; 5, Xigaze group flysh; 6, Gangdise plutonic belt; 7, folded Mesozoic series of the Lhasa block (including Takana red beds); 8, Lingzizong volcanic formation; f, schematic cross-section through the BNS; 9, Middle to Upper Jurassic flysh and slates; 10, Middle Cretaceous volcanics and red series; 11, ophiolite series; 12, Jurassic limestone and slates. g, Lead isotope diagram for the ITS magmatic section (triangles) and BNS (circles) ophiolites. The isotopic composition of the three points above the main field of the Donqiao ophiolite is due to alteration processes. The reference line is the geochron.

belonging to the genus *Protopodocarpoxylon* (gymnosperms, Cheirolepidiaceae) have been discovered that are always associated with Albian orbitolinids. This genus, which is widely represented in the Lower Cretaceous of western Europe, northern Africa and south-east Asia, has never been recorded in India or other south-Gondwana areas. Its distribution seems to fit palaeolatitudinal boundaries between 0 and 35° N. All the numerous fossil woods sampled from the Lhasa block show the presence of growth rings suggesting a palaeolatitude not too close to the Equator during Albian and Aptian times, in agreement with palaeomagnetic results. Our observations confirm previous work⁹.

The discovery of abundant palynomorphs in a level from the latest Triassic (Rhaetian) north of Lhasa suggests that an equatorial or tropical latitude was reached by the Lhasa block as early as late Triassic. The flora indicates very close relationships with the equatorial (and north equatorial) province and lacks any significant Indian taxa. Most of the taxa are commonly found in Europe and the Caucasus and more recently in Szechuan province¹⁰. The latter find suggests that not only the Lhasa block, but also the south Chinese block were situated near the Equator or not far from the tropical area as early as late Triassic^{11,12}. Somewhat surprisingly, there seems to be no unambiguous palaeontological proof of the Gondwanian origin of the Lhasa block as doubts can be cast on the significance of the Damxung-Linzu tillites. Examination of these 'tillites' is not sufficient to demonstrate that they are a glacial sediment or

alternatively rift infill. If they are indeed late Carboniferous glacial tillites, they imply a climate and latitude in striking contrast with the early Permian climate and latitude implied by the Cathaysian flora which has been discovered in the Lhasa block, and which does not contain a single Gondwanian element⁹. This indicates that if the Lhasa block indeed originated as a Gondwanian microcontinent it had detached from Gondwana as early as early Permian.

The tectonic environments of the ITS and BNS sutures are quite distinct. To the north of the ITS, the southern part of the Lhasa block can itself be divided into three zones^{1,13}. North of the Gangdise (Transhimalayan) plutonic belt, a Precambrian continental basement and its Mesozoic cover have been tightly folded in the Cretaceous with east-west trends and vertical cleavage^{1,14,15}. Within the southern part of the Gangdise belt, amphibolites, meta-pillow lavas and meta-cherts may be associated with a meta-ophiolitic suite. A thin Triassic to Lower Cretaceous sequence with low metamorphic grade occurs to the south of Lhasa. This sequence displays at least three phases of deformation: small scale (<10 m) isoclinal folds (north-south stretching lineation, slaty cleavage (S1) with southward vergence, increasing in intensity towards the ITS) are followed by a second phase, with larger (>100 m) folds (fanlike cleavage (S2) and vertical axial planes with undecided vergence). However, no unconformity which would allow precise separation of these two phases has been observed and they may be pulses of a single event. The second phase was over when the

unconformable Lingzizong volcanics were deposited. Tectonic and stratigraphical observations suggest that the Gangdise belt intruded a continent-ocean margin, leaving remnants of continental crust to the north and of (Triassic?) oceanic crust to the south. The associated Lingzizong volcanics, which span possibly from the Palaeocene to recent times^{7,8}, have themselves undergone a third phase of crustal shortening, with long wavelength folds, and both high and low angle thrust faults with small (less than a few kilometres in size) displacements.

South of the crystalline Gangdise belt, the Xigaze series may be both transgressive on the plutons to the north, and conformably deposited on the Tsangpo ophiolites to the south. The bulk of the Xigaze group consists of a thick turbidite sequence earlier thought to represent a fore-arc basin (see refs 1, 14). It has suffered east-west folding with clear southward vergence and exhibits steeply dipping cleavage and shortening of at least 30%. The ophiolite sequence on which the Xigaze formation rests has been described by Nicolas *et al.*³. Using radiolaria, Marcoux *et al.*¹⁶ have determined an age of 110 Myr for the cherts overlying the pillow lavas. Although a complete sequence from the pillows and radiolarian cherts to the Xigaze series has been observed near Tiding¹⁶, ophiolitic massifs are generally bounded by thrust or strike slip contacts and are intensely deformed. New (1982) observations have revealed important tectonic zones within the Xigaze formation and throw doubt on the previously suggested continuity of this formation between the batholith and the ITS ophiolites¹. It has already been pointed out that a very late compressive phase, postdating the Oligo-Miocene(?) Liuxu conglomerate, has in some places led to a northward backthrusting of the ophiolites. The tectonics of the Upper Cretaceous (?) *mélange*, Triassic flysch and autochthonous Indian series south of the ITS involve four shortening phases, with intense isoclinal folding and southward thrusting and symmetrical folding with steeply dipping cleavage and varying vergence between the Upper Cretaceous and the Eocene¹. This complex history of superimposed deformations may be consistent with an Upper Cretaceous to Palaeocene southward obduction of the ophiolites on top of India (see ref. 37). Alternatively, it could be related to progressive deformation within an accretionary prism at different depths. Post-Eocene deformation is mostly related to strike-slip faulting.

The tectonic style of the BNS is rather different. The suture zone has been studied in particular near Anduo and between Gyanco and Donqiao. There, an ophiolitic nappe is thrust over a thick series of Middle to Upper Jurassic sandstones and black slates. This series is locally strongly folded, slightly metamorphosed and displays a steep cleavage with variable dips both to the south and to the north. The ophiolites are in turn locally tectonically covered by a metasedimentary nappe of Palaeozoic (?) rocks. All these units are unconformably overlain by a Middle Cretaceous volcanic and red detrital formation. These formations have undergone isopaque (constant thickness) folding, with N140° axes, show a cleavage with undecided vergence, and thrust faulting accompanying late emplacement of the ophiolites on the red beds. Although the ophiolites have been severely deformed, they exhibit all the traditional components of the suite (pillow-lavas), a sheeted dolerite complex overlying garnet-bearing amphibolites, isotropic to layered gabbro, troctolites, wehrlites to dunites and serpentinized residual harzburgites containing chromite rich bodies. The ophiolites have been reworked in a grey neritic limestone of Upper Jurassic age. Moreover, in Donqiao, the ophiolites seem to be covered in turn by a transgressive marine detrital Upper Jurassic to lowermost Cretaceous series.

The Quantang block, north of Anduo, is less well known, as observation there was far less extensive. It seems to involve rather simple, broad, folding of dominantly neritic Jurassic sediments. Rapid surveys and inspection of the geological map of China seem to indicate that plutonic and volcanic formations are far less abundant to the north of BNS, which, of course, raises questions as regards the polarity of the former subduction zone. A southward dipping subduction would be favoured by

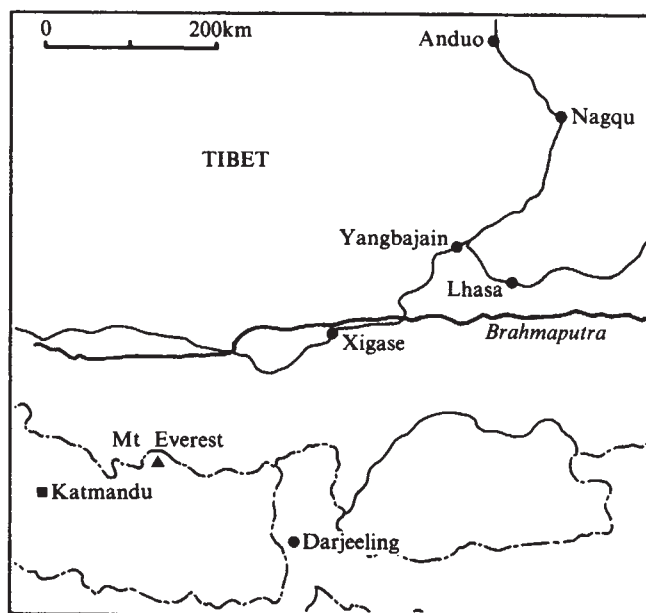


Fig. 2 Map showing the relationship of southern Tibet to the Himalaya. The place names given are those used by the French-Chinese three-year geoscience cooperation programme.

the scanty presently available data, opposite to what was previously thought (see ref. 6).

The analysis of lead isotopes in samples of ophiolites from the ITS and BNS sutures reveals striking differences (Fig. 1). The Pb isotopic composition of the magmatic sequence from ITS falls in the field of dredged samples from the Indian Ocean Ridge¹⁸. This may indicate a genetic relationship between the sub-Indian Ocean mantle and the ophiolites. A U-Pb total rock age of 119 ± 25 Myr has been obtained from the magmatic section of the ophiolite near Xigaze. In contrast, rocks from BNS show higher 207/204 ratios and resemble ophiolites from the eastern Mediterranean¹⁹. This is compatible with the Bangong-Nujiang ophiolites have formed in a small back arc or interarc basin, as indicated by the palaeolatitudinal studies reported above.

The ultramafic part of the Tsangpo ophiolite has an isotopic signature that is similar to that of the Donqiao pillow-lavas with higher 207/204 ratios than in the magmatic section. This suggests that the two parts of the Tsangpo ophiolite may not be cogenetic.

The volcanic environment of the two sutures is also contrasted. Volcanic rocks are very abundant in the south of the Lhasa block. They occur as ignimbritic tuffs in the Middle Cretaceous Takena formation and form most of the 1,500-m thick Lingzizong formation, with piles of andesite flows and rhyolitic ignimbrite flows. This thick calc-alkaline sequence contrasts with the less abundant mostly basic volcanic rocks of BNS. Only a few ignimbrite flows are observed there. In Nagqu, volcanic rocks overlie the black Jurassic slates and seem to postdate the Cretaceous red beds in Anduo. These red beds are sometimes thrust over the basalts and andesites. Thus, the volcanic environments of ITS and BNS appear to be suggestive of Andean and island-arc subduction respectively, although the age of the northern volcanics remains to be determined.

Plutonic rock and basement gneisses have been sampled in a fairly systematic way to obtain geochronological data (U-Pb: accessory minerals) and to identify the source regions of the granitoid magmas (Nd, Sr and Pb initial isotopic compositions). The ages at which the basement material was transformed into gneisses, although poorly defined because of the heterogeneity of the inherited Pb components, appear to be synchronous with the plutonic activity. Inherited Pb-components in zircons yield

Fig. 3 Wide-angle seismic refractions from the Moho along the section shown in Fig. 2 reveal a complex Moho topography (after refs 20–22). First arrivals are picked at the dashed lines, providing the Moho topography. Particularly noteworthy are the 20-km step in the Moho near ITS and the presence of two superimposed Mohos at the latitude of Yangbajain. The profiles result from two shots in Angren and the Shi Lin Co (about 100 km outside of the figure to the west) recorded in fans along the main road from Quxu to Nagqu. No vertical exaggeration.

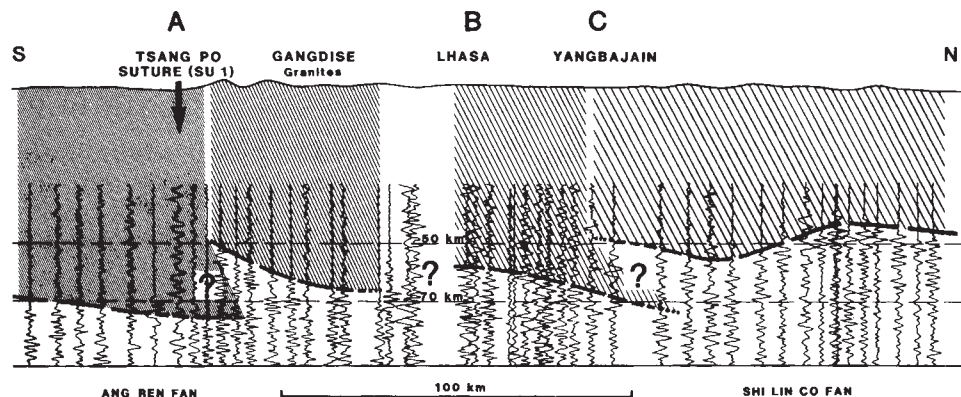
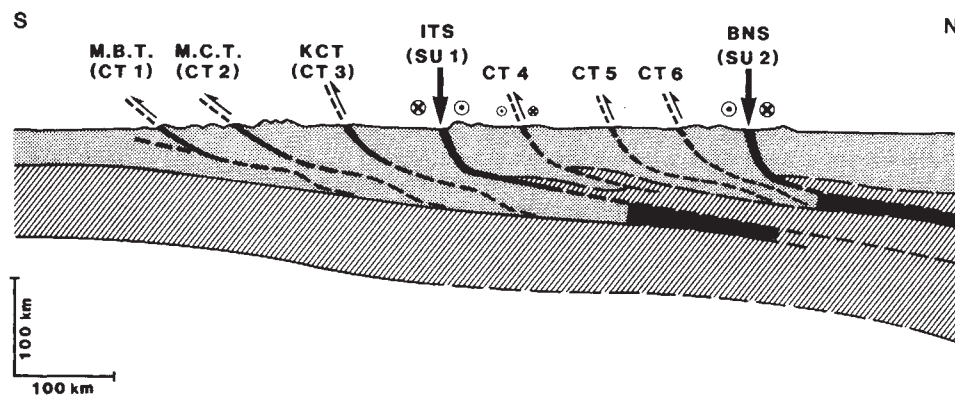


Fig. 4 A model for the structure of the lithosphere in the Tibet-Himalaya. The role of obduction related sutures (Indus Tsangpo suture or ITS, Bangong-Nujiang Suture or BNS) and of continental thrusts on the Indian plate (main boundary thrust, main central thrust, Kangmar thrust labelled CT1 to CT6) and in the Lhasa block (CT4 to CT6) corresponding to possible thrusts near Yangbajain, Gulu and South of Anduo) is emphasized. Continental and oceanic crusts are shaded in light and dark grey respectively. The subcrustal lithosphere is ruled. Older sutures and continental thrusts are rotated into the vertical and reactivated as strike-slip faults.



minimum ages up to 2,200 Myr in the Everest area and also Precambrian ages in the Yangbajain and Anduo areas. Clearly, all crustal blocks are made of old basement materials. The leucogranites of the High Himalayas, and those intruding the Tethyan sediments further north, yield ages of 15–30 Myr and seem to be purely anatectic. The Gangdise plutonic belt comprises numerous bodies of gabbro, diorite, granodiorite and granite which have been intruded from 95 to 40 Myr ago. Initial Nd, Sr and Pb isotopic compositions show large heterogeneities and plot along trends interpreted as a mixture of mantle-derived component(s) with continental crust materials. The volcanic rocks of the Lingzizong formation have the same isotopic characteristics implying a similar origin. In both cases, the crustal components appear to be dominant. Farther north, the Yangbajain, Gulu and Anduo granitoid belts yield ages around 50, 100 and 100 Myr respectively. The Pb-isotopic composition of these rocks is compatible with an origin by partial melting of the basement materials. From the point of view of geochemistry, the Gangdise belt looks like a subduction related complex built on continental crust, similar to the Sierra Nevada batholith, whilst other intrusives farther north in Tibet are anatectic granitoids.

Thus, the available information suggests that the ITS corresponds to an Andean subduction environment, which has been terminated between the Upper Cretaceous and the Eocene by the collision of India with the Lhasa block, acting as the southern margin of Asia. On the other hand, the BNS is different and a likely interpretation at this stage involves an island-arc subduction environment, which has been terminated in the Upper Jurassic to Lower Cretaceous by a collision between the Lhasa block and the Quangtang block, then the southern margin of Asia.

Crustal repetition

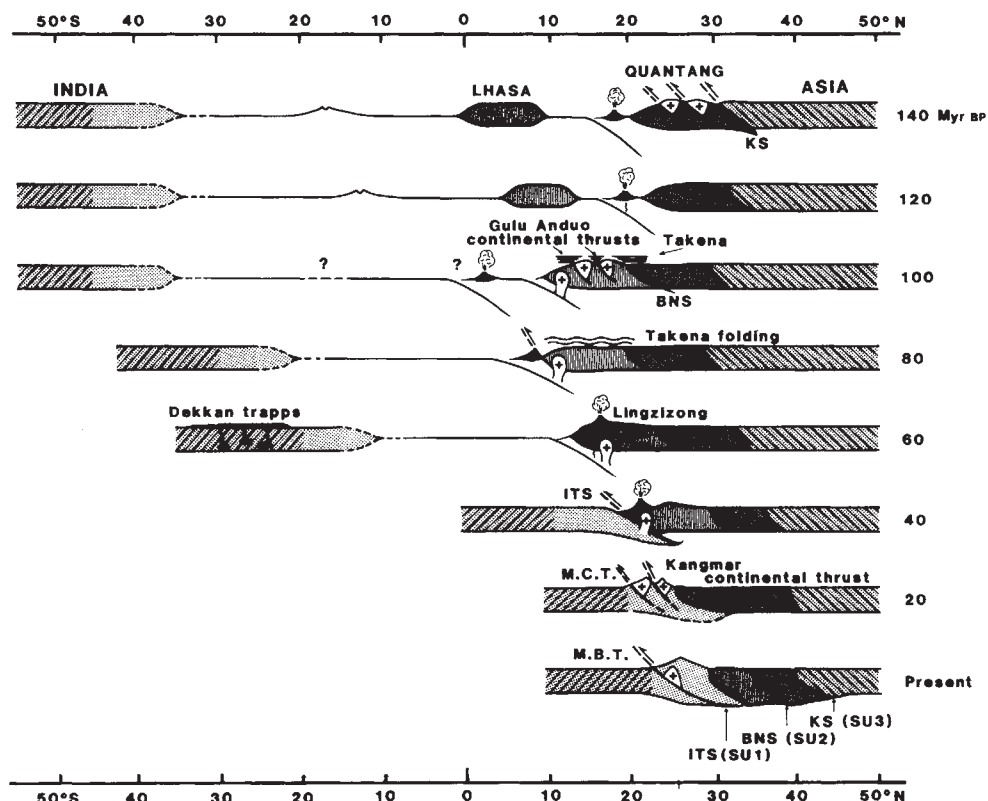
A 300-km long north-south seismic section has been obtained by Hirn *et al.*^{20–22}, using critical reflection profiling on the Mohorovicic discontinuity, complemented by fan profiles. The detailed data and interpretation are given elsewhere^{20–22} and only the major features are summarized here (Fig. 3). The

section reveals a thick crust (between 50 and 70 km) with a complex structure. An outstanding feature is a 20-km step in the Moho precisely below the surface trace of the ITS which is consistent with strike-slip reactivation of a major thrust contact. The data do not allow resolution of whether or not the Lhasa block is actually underlain by Indian crust. Crustal repetition occurs along parts of the section, particularly north of Yangbajain: there two superimposed Mohos are observed with the lower crustal member being ~20 km thick. Another 20-km step in the Moho, very similar to that observed below the ITS has been found near Anduo below the BNS (outside of the range of Fig. 4, see refs 20–22). Thus, the Tibetan Moho displays a complex topography with slopes and faults and in places implies crustal repetition (although the two crustal members are not necessarily complete) by continental thrusting.

It is now widely accepted that the structure of the High Himalayas is associated with a major intracrustal thrust, the MCT, with the northern Himalayan crust thrust southwards. The throw on the MCT is not yet known but may be very large. It seems that the MCT is no longer the active thrust but that tectonic activity has jumped southward, with a new continental (crustal) thrust, the MBT, presently active^{23–25}. Intense shear along the MCT within the lower structural levels of the Tibetan slab is linked with the genesis of the 20-Myr old anatectic Himalayan leucogranites. A detailed field study of the Lhozag-Kangmar-Lhagoi-Kangri belt of granite plutons, about 100 km to the north of the High Himalayas²⁶, also implies that a major continental thrusting episode occurred there, possibly leading to crustal thickening, and associated with a high grade metamorphic episode contemporaneous with thrusting on the MCT (around 20 Myr).

The gneisses around Yangbajain, about 100 km north of the ITS, have a Precambrian primary age but have undergone a young metamorphic episode at around 50 Myr. This is also the age of the Yangbajain anatectic granites. The U-Pb systematics of zircons imply that these gneisses have been formed at a depth of ~15 km. They may have been thrust over the Cenozoic Lingzizong volcanic formation and thus be related to another intracrustal fault with 15 km of vertical offset. It may be sug-

Fig. 5 An attempt to reconstruct the evolution of the Lhasa block in relation to the Indian and Asian continents since the beginning of the Cretaceous, at 20-Myr intervals. The blocks are located in latitude in agreement with palaeomagnetic (after 100 Myr) and palaeontological (before 100 Myr) data. Shortening of the Lhasa and Quantang blocks by a factor of two and of the northern margin of India by about 1,000 km have been included. Closing of sutures, activation of continental thrusts and related granite formation, Tarena deposition and folding and Lingzizong volcanic activity have been emphasized. Controversial aspects are, of course, present such as the origin of Tarena folding by collision with an island arc (see text for another possibility).



gested that the genesis of granitoid rocks in Tibet is related to large-scale intracontinental thrusts (leading to crustal doubling or thickening) and that, for instance, the Gulu and Anduo belts are the signature of older (100 Myr old) thrusting events. This would be a major means of crustal thickening in Tibet: a picture emerges where two sutures, both related to major continental collisions, separate thickened continental blocks in which thickening (crustal repetition) is related to as many as six major thrusts (MBT, MCT, Kangmar, Yangbajain, Gulu, Anduo from south to north; see Fig. 4). These continental thrusts might result from post-collisional continuing convergence.

Some of the authors of this article are, however, reluctant to accept Yangbajain as the site of a major southward verging Tertiary intracontinental thrust. Some structural observations show that between Yangbajain and Nagqu deformation of late Mesozoic to Cenozoic age is characterized by steep cleavage dipping mostly south and, locally, horizontal stretching lineations, and thus presumably indicative of large scale strike slip motion, dominantly in an east-west direction. The relative importance of thrusting and strike slip faulting with respect to crustal shortening in Tibet has yet to be analysed in detail.

Kinematic evolution

The stratigraphical and tectonic observations, together with the isotopic age determinations and palaeomagnetic data reported here allow us to propose a geodynamical model for the evolution of blocks which are now part of the Indian-Asian continent over the past 120–140 Myr. The time and space distribution of our data is, of course, still far from complete and the interpretation that we propose is not unique at all periods. The present model (Fig. 5) includes the possibility of lithospheric shortening by continental thrusting and is controlled in latitude by palaeomagnetic and palaeontological data. It begins around 140 Myr ago. The Quantang block was sutured to Asia along the Kokoxili suture ~200 Myr ago and some crustal shortening may subsequently have occurred on (hypothetical) continental thrusts. Around 140 Myr ago, a small ocean basin between the Quantang and Lhasa blocks closed along the BNS (its size at 140 and 120 Myr has been exaggerated on Fig. 5). Continental shortening followed along the Gulu and Anduo thrusts and continental red beds were deposited over this southern margin

of the Asian continent. Folding of the red series may have occurred around 80 Myr or later. Its origin is still puzzling and it may either be due to interaction with a hypothetical island arc (which would have to have been completely obliterated subsequently), or be linked with the subduction process (Andean folding) or be related to the obduction and collision themselves. At this time, the Lhasa block was at low latitudes. The Lingzizong arc volcanics were erupted between 60 and 45 Myr, at which time the subduction zone was blocked and obduction occurred along the ITS. Continental shortening then occurred in the Yangbajain (40 Myr), Kangmar and MCT areas (20 Myr), recently jumping to the MBT. An unknown length of Indian continental lithosphere may also have been thrust under the MCT. Our schematic representations suggest that it may be up to 1,000 km long as suggested by Argand in 1924²⁷.

Geodynamical consequences

Our observations have important geodynamical implications: first, they may provide constraints on the mechanism of continental accretion and evolution, second they can tell us something about the driving mechanism of plate tectonics.

We have suggested that there may be as many as two continental collisions and six continental thrusts between 26 and 33° N at the longitude of Lhasa. There are two more collisions (suture zones) and possibly several continental thrusts north of the area that we surveyed, between 33 and 40° N. Each one of these tectonic features leads to the disappearance of some continental surface, to crustal thickening and to high elevations. As time passes, erosion will destroy high mountains and plateaus, thin the crust and bring it back to its 'normal' 35-km continental thickness. In the process, continental crust will have been removed not only along horizontal dimensions (surface area) but also along the vertical dimension (volume loss). The eroded material will, of course, be redeposited elsewhere, although much of it is likely to be returned to the mantle in nearby active subduction zones. A very quick and crude estimate of the surface and volume loss in Tibet over say, the past 150 Myr can be made: over a 2,500 km long front, from the Pamir to the Assam syntaxis, blocks that now extend over 1,500 km from north to south with a double continental crustal thickness may have originally extended over as much as 3,000 km. The total surface

loss is of the order of $4 \times 10^6 \text{ km}^2$ (or between 2 and $3 \times 10^{-2} \text{ km}^2 \text{ yr}^{-1}$ in average surface loss) and the final volume loss will amount to over $100 \times 10^6 \text{ km}^3$, or between 0.5 and $1 \text{ km}^3 \text{ yr}^{-1}$ on average. It is interesting to compare this value with the rate of possible sediment loss in subduction zones, which is of the order of $1\text{--}2 \text{ km}^3 \text{ yr}^{-1}$ (taking average sediment thickness, length of trenches and subduction velocity of 0.5 km, 40,000 km and 5 cm yr^{-1} respectively). Thus, continental collision acts as a significant limiting factor for continental growth (which is itself related to volcanic and plutonic activity in subduction zones).

Nevertheless, continental collision has enlarged the Asian continent by adding allochthonous continental fragments to it. The older sutures are the remnants of continental collisions related to previous accretion of allochthonous blocks to Asia since the beginning of the Palaeozoic⁶. The situation encountered in western North America²⁸ is reminiscent of what we observe in Tibet. The origins of each individual block may be quite diverse, as shown by the analysis of faunas and floras. The Lhasa block for instance is a Cathaysian block and not necessarily of Gondwanian origin. Together with much of Indochina, the Lhasa block may have been part of Pacifica²⁹. Thus the Asian continent actually results from the juxtaposition of many blocks of diverse origins and is a typical continental mosaic.

The sutured edges of previously accreted continental blocks will induce large-scale heterogeneity and anisotropy of deformation when a new block is accreted through collision. Those earlier sutures which are favourably oriented with respect to the principal compressive stress due to the new collision will be reactivated, often as large strike-slip faults³⁰. Some of the major strike-slip faults described by Tapponnier and Molnar³¹ in Tibet are rejuvenated sutures. This is the case for large segments of most sutures described here. It is also true for other parts of China, and in Tibet for several continental thrusts (such as the ones near Yangbajain and Gulu, for instance). However, active faults resulting from a collision need not necessarily all be reactivated block boundaries: the most efficient way to join two reactivated strike slips faults may in cases be to create a new fault through some particular block in the mosaic. Still, an active tectonic phase is in some ways guided by tectonic features resulting from previous phases. If the mode of accretion of the Asian continent is to be regarded as the rule rather than the exception for continental accretion, then earlier continental collisions are important in establishing the tectonic grain of a future continental collision.

Following the work of Forsyth and Uyeda³², many authors have considered the pull of descending slabs at oceanic subduction zones as the main force acting to drive plate motion. Other forces, such as the so-called 'ridge push', gravity sliding and shear coupling to the asthenosphere have been regarded as less important and in any case not resolvable. However, it seems clear that slab pull cannot be the leading force in the case of

the relative motion of the Indian plate with respect to Asia. Subduction along the ITS stopped at least 45 Myr ago and since then, there has been no oceanic lithosphere left between India and Asia. Continuing slab pull beneath Java and Sumatra is not likely to be sufficient to counteract alone resistance to convergence in the Himalayas. Yet, intracontinental motion continues on the MCT and Kangmar CT around 20 Myr, and now on the MBT, at a rate of about 2 cm yr^{-1} (ref. 33). The total motion between India and Asia is 5 cm yr^{-1} (ref. 31).

Continental shortening is also taken up along sets of large strike-slip faults^{31,34}. Models of continental delamination (see ref. 35) cannot account for the continued penetration of India either, and other boundary forces must be sought. The northward motion of India lasted since the Jurassic and the subcontinent was subjected to considerable tensile stress from maybe as early as 110–60 Myr ago when the Dekkan traps were produced. Recent geochemical analyses suggest that the traps result from the fusion of the continental lithosphere and do not involve deeper components³⁶. This observation seems difficult to reconcile with any significant contribution from the ill-named 'ridge push' force. Moreover, before India was rifted away from Gondwana, other continental fragments, rifted earlier either from Gondwana or elsewhere, were already 'attracted' towards Asia in a dominantly northward direction and collided with it³⁰.

Thus, there is a long history, probably more than 200 Myr long, of polarized accretion of blocks to the southern margin of the Asian continent. A similar long-lived polarization of motion is suggested by the study of allochthonous terranes accreted to North America and sheared along the San Andreas–Denali strike-slip system (see ref. 28). These observations seem to imply the persistence over several hundred million years of large-scale convection currents, more or less stationary with respect to the mantle, and coupled to the lithosphere. A possible mechanism is gravity sliding over the hot ascending limb of such a large convection cell. The initial breakup of Gondwana, the motion of small blocks and then of India, the successive collisions and present continental shortening would thus all be due to northward gravity sliding over a large hot mantle zone lasting at least 200 Myr. This zone may well correspond to the Atlantic–African geoid high, as recently argued by Anderson¹⁷. The trend of the southeastern part of this geoid high, associated with the Réunion, Kerguelen and Crozet hotspots, is compatible with the observed direction of polarized motion, that is perpendicular to it.

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1. Tapponnier, P. *et al.* *Nature* **294**, 405–410 (1981); *Résultats de la coopération franco-chinoise au Tibet (mission 1980)* (eds Mercier, J. L. & Guangqin, Li) (CNRS, Paris, in the press).
2. Tapponnier, P., Mercier, J. L., Armijo, R., Tonglin, H. & Ji, Z. *Nature* **294**, 410–414 (1981).
3. Nicolas, A. *et al.* *Nature* **294**, 414–417 (1981).
4. Pozzi, J. P. *et al.* *Nature* **297**, 319–321 (1982).
5. *Colloque Franco-Chinois sur la Géologie de l'Himalaya*, Abstr. Guilin (1982).
6. Chang Chenfa & Cheng Hsian, *Sci. Sinica*, **16**, 257–265 (1973); in *Proc. Symp. on Qinghai-Xizang (Tibet) Plateau*, (Gordon & Breach, New York, 1981).
7. Westphal, M., Pozzi, J. P., Yaoliu, Zhou, Lisheng, Xing & Xian Yao, Chen, *Geophys. J. R. astr. Soc.* **73**, 507–521 (1983).
8. Maluski, H., Proust, F. & Xuchang, Xiao, *Nature* **298**, 152–154 (1982).
9. Hsü, J. *Scient. Geol. Sinica* **10**, 323–331 (1978).
10. Schang, Y. *Sih int. palyn. Conf.*, (Cambridge University Press, 1980).
11. Buffetaut, E. *Bull. Soc. géol. Fr.* **23**, 587–593 (1981).
12. Ingavat, R. & Janvier, P. *Geobios* **14**, 725 (1981).
13. Burg, J. P., Proust, F., Tapponnier, P. & Guoming, Cheng, *Ecol. Helv.* (submitted).
14. Shackleton, R. J. *struct. Geol.* **3**, 97–101 (1981).
15. Bally, A. W. *et al.* *U.S. geol. Surv. Open File Rep.* **80-501** (1980).
16. Marcoux, J. *et al.* *Ofolit Suppl.* **6**, 31–32 (abstr.) (1981).
17. Anderson, D. *Nature* **297**, 391–393 (1982).
18. Dupré, B. & Allègre, C. J. *Nature* **303**, 142–146 (1983).
19. Hamelin, B., Dupré, B. & Allègre, C. J. *Earth planet. Sci. Lett.* (in the press).

20. Hirn, A., Jobert, G., Wittlinger, G., Zhongxin, Xu & Enyuan, Gao, *Ann. Géophys.* (submitted).
21. Hirn, A., Nercissian, A., Zhongxin, Zu, Deyan, Lu & Jiwen, Teng, *Terra Cognita* **3**, 267 (1983).
22. Hirn, A. *et al.* *Nature* **307**, 23–25 (1984).
23. Barazangi, M. & Ni, J. *Geology* **10**, 179–185 (1982).
24. Mattauer, M. C. R. *hebd. Séanc. Acad. Sci., Paris* **296**, 481–486 (1983).
25. Seeber, L., Armbruster, J. G. & Quittmeyer, R. in *Zagros-Indukush-Himalaya Geodynamic Evolution* (eds Gupta, H. K. & Delany, F. M.) 215–242 (1981).
26. Burg, J. P., Guiraud, M. & Guoming, Cheng, *Earth planet. Sci. Lett.* (submitted).
27. Argand, E. C. *r. Congr. géol. int.* **13**, 171–372 (1924).
28. Ben Avraham, Z., Nur, A., Jones, D. & Cox, A. *Science* **213**, 47–54 (1981).
29. Nur, A. & Ben Avraham, Z. *J. geophys. Res.* **87**, 3644–3661 (1982).
30. Tapponnier, P., Mattauer, M., Proust, F. & Cassaigneau, C. *Earth planet. Sci. Lett.* **52**, 355–371 (1981).
31. Tapponnier, P. & Molnar, P. *Nature* **264**, 319–324 (1976); *J. geophys. Res.* **82**, 2905–2930 (1977).
32. Forsyth, D. & Uyeda, S. *Geophys. J. R. astr. Soc.* **40**, 465–474 (1975).
33. Armijo, R., Tapponnier, P., Mercier, J. L. & Tonglin, Han, *EOS* **63**, 1093 (1982).
34. Molnar, P. & Tapponnier, P. *Science* **189**, 419–426 (1975).
35. Bird, P. *J. geophys. Res.* **84**, 7561–7571 (1979).
36. Allègre, C. J., Dupré, B., Richard, P., Rousseau, D. & Brooks, C. *Earth planet. Sci. Lett.* **57**, 25–34 (1982).
37. Burg, J. P. thesis, Montpellier Univ. (1983).
38. *Geological map of the Tibetan Plateau*, 1/1,500,000, Chengdu, China (1981).

Crustal structure and variability of the Himalayan border of Tibet

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Reversed critical wide angle reflection profiles of the crust-mantle boundary south of the Yarlung Zangbo suture in Tibet reveal a deep (70 km) Moho extending north from the High Himalayas, whilst to the south the Moho is 15 km higher. Even further south, reflections from 35 km depth may correspond to the Moho of peninsular India.

IN September–October 1981 seven charges ranging from 2 to 10 tons were detonated in two lakes and at one drill-hole shotpoint between the High Himalayas and the Yarlung Zangbo Jiang as part of the French–Chinese geoscientific exploration of southern Tibet. Forty portable tape recording seismic stations were laid out to cover a 500-km long east–west line with a system of reversed and overlapping profiles, together with auxiliary profiles (Fig. 1). Twelve additional stations in Nepal tested the propagation through the High Himalayas. Details of the method, experiment and interpretation are presented elsewhere^{1–3} and partly summarized below.

Interpretation of critical reflections

Critical reflections recorded from the crust-mantle boundary (Fig. 2a) are exceptionally clear when compared with former experiments on thick crusts of complex origin^{4–9}, a result partly

attributable to propagation along the strike of geological units. The crust-mantle boundary can be modelled (using synthetic seismograms¹⁰) as a transition zone about 12 km thick at 75 km beneath the present-day surface (4,500 m mean elevation). Such a large crustal thickness was unexpected so near to the High Himalayas. In the upper crust strong variations in seismic response along the Tibetan line indicate significant structural variation, but reflectors at mid-crustal depth can be detected on most profile sections. Crustal mean velocity, derived from reversed observations of the Moho reflection, is of the order of 6.3 km s^{-1} , with upper and lower crust mean values around 6.0 and 6.4 km s^{-1} , respectively. The slope of the spectral ratio and the comparison of overall amplitudes of waves reflected respectively, on a mid-crustal interface and on the Moho provide a significantly high value for P-wave attenuation in the lower half of the crust of the order of 10^{-2} . Another peculiar feature

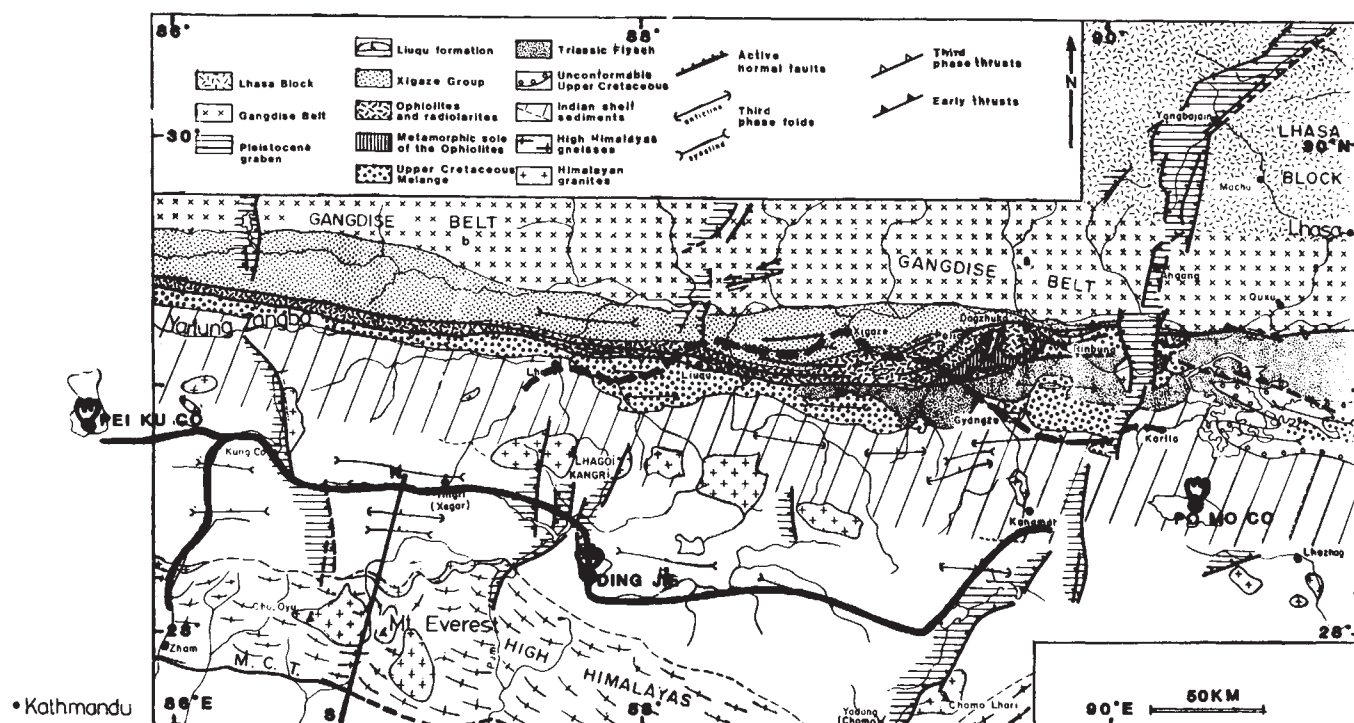


Fig. 1 Shotpoints in Tibet in 1981: Pei Ku Co, Ding Jie and Po Mo Co. Heavy lines are the location of profiles of seismographs with 10 km spacing. Geology is from Tapponnier *et al.*¹⁸. S–N is the position of the crustal section of Fig. 3.

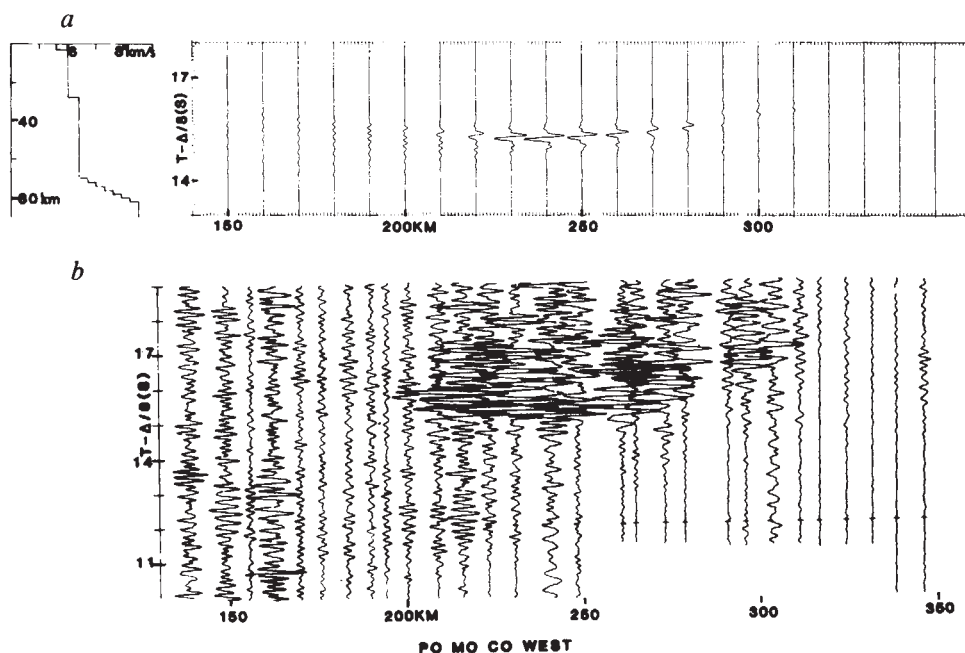


Fig. 2 *a*, Synthetic record section for a 12-km thick transition from crust to mantle velocity around 75 km beneath the surface. *b*, Shots Po Mo Co recorded on the east-west in-line profile. True amplitude record section reduced to 8 km s⁻¹ velocity. Note the prominent burst of energy returned between 200 and 300 km distance and 15 and 16 s reduced time due to reflection at the Moho.

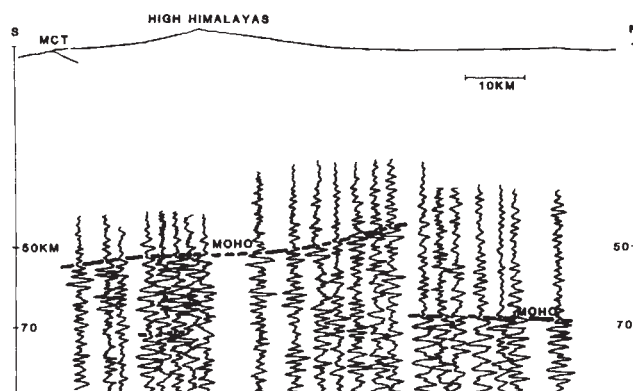


Fig. 3 Fan-profile to the west from shotpoint Dingjie. Correlated phases at these shotpoint distances from 180 to 300 km correspond to reflections at the crust-mantle boundary (see Fig. 2). Reflection times are corrected to a constant offset of 200 km, so that the time-section is directly transformed in a depth-section (S-N on Fig. 1) across the High Himalayas under the assumption of negligible variation of mean crustal velocity along it.

derived from the east-west reversed profile is a value of Pn velocity greater than 8.5 km s⁻¹.

North-south crustal profile

North-south crustal variation at the level of the Moho has been investigated using the relative arrival times of the reflections at critical-wide angle, that is, at 200–300 km range, on fan profiles in southern Tibet and across the High Himalayas to Nepal. Figure 3 shows a topographical cross-section of the deep crustal interfaces west of Mt Everest, obtained from a time-section of reflected waves at their mirror position. This section has been corrected for different offset distances and transformed into a depth-section assuming negligible variation of average crustal velocity. The deep Tibetan Moho is seen to extend to southwards to within a few tens of kilometres of Mt Everest. Another crust-mantle interface, probably dipping northwards, is seen at 55 km depth under the High Himalayas and partly on top of the Tibetan Moho. The mantle is deeper than expected beneath and south of the High Himalayas. Nevertheless, the depth to the mantle can be reconciled with the observed values of the gravity field^{11,12} by taking into account the abnormal thickness

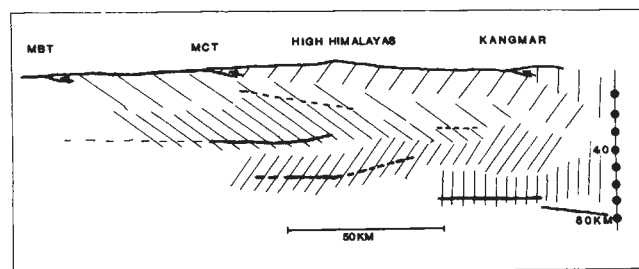


Fig. 4 Sketch of elements of seismic structure across the High Himalayas. Dashed line: interface between upper and lower crustal velocity material. Solid line: interface between crust and mantle velocity material. Loose hatching: upper crust layers. Their superposition is supposed to occur along flat thrusts at the base of the overriding unit. Tight hatching: lower lithospheric units. These are comprised of the lower crust, Moho and a sole of uppermost mantle material and are assumed to be brought to the present position, attested by the position and shape of the Moho marker, as a result of thrusting and superposition along levels of decoupling in the middle or lower crust and at shallow depth in the uppermost mantle. Possible correspondence of upper and lower units is indicated by hatching of same direction. MBT, Main Boundary Thrust; MCT, Main Central Thrust.

of the low velocity upper half of the crust and the density deduced from the intermediate velocity value at the crust-mantle transition. However, the crustal section derived from explosion seismology no longer supports a continuous, smoothly north-dipping trend for lithosphere features beneath the Himalayan border of Tibet (a model which was suggested by gravity data and was consistent with a simple underplating of the crust causing a doubling of crustal thickness to the North¹³), nor slicing and superposing of crustal units on top of a decoupled, smoothly depressed Moho^{14,15}.

Evidence for further sharp topographical variations of the Moho to the north is derived from a fan profile between the High Himalayas and the Yarlung Zangbo. Here the crust-mantle transition zone appears to be composed of reflecting elements which may overlap each other at depth intervals of the order of 10 km (ref. 3).

To the south, and possibly on top of the Everest Moho (55 km depth), a reflector which could be another segment of crust-mantle interface is shown by a profile in Nepal through Kathmandu, in line with the western shotpoint in Tibet. Here the depth difference between these interfaces is of the order of

20 km so that the shallower one could be the Moho of peninsular India at 35 km depth².

Conclusions

Figure 4 summarizes the explosion seismology data by projection onto a cross-section from the Main Boundary Thrust (the southern extremity of the lower Himalayan domain) to the Yarlung Zangbo. This figure suggests that the observed thickening of the crust from north to south is not due to a smoothly north-dipping Moho. The amplitude of the steps is significantly smaller than normal crustal thickness, suggesting that the crustal doubling is not the result of the superposition of two whole normal crusts on each other, but rather the result of separate doubling (by decoupling and thrusting) of the upper and lower crustal layers (plus Moho). The resulting structure would be consistent with the observation that the 70-km thick Tibetan

crust can be divided into two layers of different mean velocities, each being twice as thick as the corresponding layers of normal crust.

The decoupling at mid-crustal depth thus implied may have occurred at the lower limit of the brittle layer or at the base of the upper, less basic layer in a compositionally layered crust¹⁶. Decoupling beneath the Moho might be related to the behaviour of a low velocity layer at a shallow depth in the mantle lithosphere¹⁷.

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1. Hirn, A., Jobert, G., Wittlinger, G., Xu, Z. X. & Gao, E. Y. *Ann. Géophys.* (in the press).
2. Lépine, J. C., Hirn, A., Pandey, M. R. & Tater, J. M. *Ann. Géophys.* (in the press).
3. Hirn, A. & Sapin, M. *Ann. Géophys.* (in the press).
4. Ocola, L. C. & Meyer, R. P. *Geophys. J. R. astr. Soc.* **30**, 199–209 (1972).
5. Ocola, L. C., Aldrich, L. T., Gettrust, J. F., Meyer, R. P. & Ramirez, J. E. *Bull. seism. Soc. Am.* **65**, 1681–1695 (1975).
6. Finetti, I., Giorgetti, F. & Poretti, G. *Boll. Geof. teor. appl.* **21**, 159–170 (1979).
7. Belousov, V. V. *et al. Tectonophysics* **70**, 193–221 (1980).
8. Kaila, K. L., Krishna, V. G., Chowdhury, K. R. & Narain, H. *J. geol. Soc. India* **19**, 1–20 (1978).

9. *Acta geophys. Sinica* **24**, 155 (1981).
10. Fuchs, K. & Mueller, G. *Geophys. J. R. astr. Soc.* **23**, 417–433 (1971).
11. Kono, M. *Geophys. J. R. astr. Soc.* **39**, 288–299 (1974).
12. Tang, B. X., Liu, Y. L., Zhang, L. M., Zhon, W. H. & Wang, Q. S. *Geological Ecological Studies of Qinghai-Xizang Plateau* Vol. 1, 683 (Science Press, Beijing, 1981).
13. Powell, C. & Conaghan, P. J. *Earth planet. Sci. Lett.* **20**, 1–12 (1973).
14. Lyon-Caen, H. & Molnar, P. *J. geophys. Res.* **88**, 8171–8191 (1983).
15. Mattauer, M. *Terra Cognita* **3**, 269 (1983).
16. Meissner, R. *J. Geophys.* **40**, 57–73 (1974).
17. Hirn, A., Steinmetz, L., Kind, R. & Fuchs, K. *Z. Geophys.* **39**, 363–384 (1973).
18. Tapponnier, P. *et al. Nature* **294**, 405–410 (1981).

Lhasa block and bordering sutures— a continuation of a 500-km Moho traverse through Tibet

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A north-south wide-angle fan-profile through the Yarlung Zangbo suture, across the Lhasa block and through the Bangong-Nujiang suture shows several significant and sharp changes in crustal thickness. Both sutures appear as vertical 20-km steps in the Moho and may have been the loci for eastward strike-slip motion of the Tibetan lithosphere.

THE Yarlung Zangbo Jiang suture zone between southernmost Tibet, part of the Indian continental plate, and the Lhasa continental block, part of Asia since before the Cretaceous, was a major target for the seismic exploration of deep structure in the 1981–82 French-Chinese joint programme. The results of the first phase (an east-west profile in the south Tibetan zone between the High Himalayas and Yarlung Zangbo suture) are reported in the accompanying article¹ and elsewhere^{2–4}. The second phase in June 1982 was intended to record a parallel line in the Lhasa block, to the north of this suture, to allow a comparison of structures. However, difficulty of access obliged us to displace it 300 km to the north, near the other limit of the Lhasa block. Consequently fan-recording was used to investigate crustal variation between these lines.

From a methodological viewpoint it is worth noting that the width of individual crustal segments between Moho disruptions thus defined is of the order of their thickness (50–100 km). As the critical distance for waves reaching the Moho is much larger (200 km) than this width, these segments could hardly have been resolved by waves propagating across them in a conventional north-south in-line shooting and recording except perhaps

by using numerous tightly spaced shotpoints. Although, in principle, vertical seismic reflection profiling of the continental crust⁵ offers the possibility of improved resolution of horizontal variations, significant attenuation in the deep Tibetan coast³ would affect high frequency signals.

Depth-section

The depth section of deep crustal reflectors presented here can be tied in with sections further south discussed in the accompanying article¹ to establish a 500-km long Moho traverse north of Kathmandu (Fig. 2). Parts of it, principally in the northern third, are provisional, as the interpretation now in progress of east-west in-line profiles may add or modify some conclusions. The depth-section is obtained under the assumption that the average crustal velocity is regionally constant. Most seismograms were obtained at constant distance, 200–250 km, from shotpoints, so the plot is that of the time-section; in some parts, however, the data are corrected for larger offset distances. The largest uncertainty in the conversion of time into depth results from lack of control of possible variations in the sharpness of the velocity contrast across the Moho along the section. In contrast to vertical

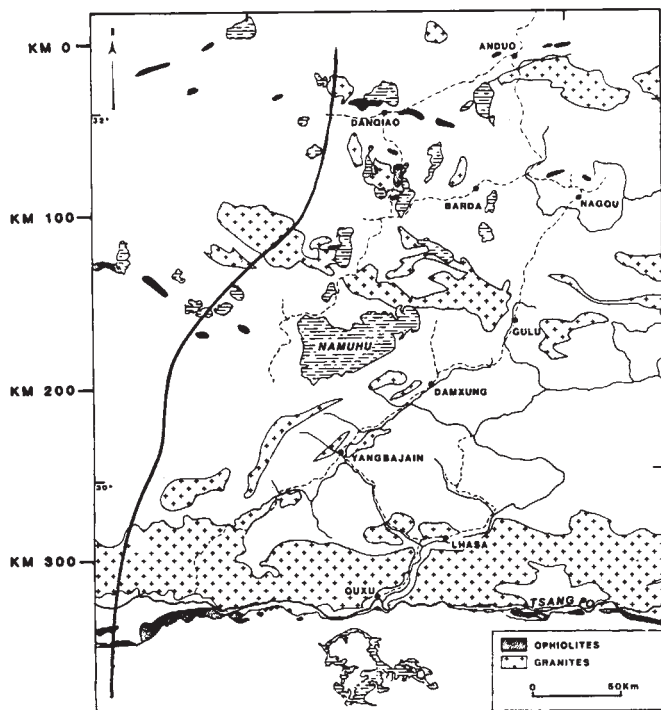


Fig. 1 Map with simplified geology, southern Tibet. The black line is the location of the seismic cross-section of Fig. 2. Data were recorded in a fan-like manner by seismic stations aligned along the main road through Quxu, Yangbajain, Gulu, Nagou and Anduo at approximately equal distances of 250 km from two shotpoints to the west near latitudes 32° N at Shi Lin Co Lake and $29^{\circ}5'$ N in drill-holes at Ang Ren. Distances (in km) from Fig. 2 are taken from the left side scale. South of km 300 the section of Fig. 2 is a composite of data discussed in the accompanying paper¹.

reflection profiling, only interfaces for which the offset of 250 km corresponds to the angle of incidence for critical reflection respond by sending back strong signals. Clear correlated onsets correspond to deep interfaces between crust and mantle material, and cannot originate as simple or multiple reflections from shallow levels or from deep levels with only small velocity contrasts.

North of the Himalayan region discussed previously¹, the 70-km deep Moho can be traced at approximately that depth until it is abruptly interrupted in the region of the Yarlung Zangbo. The Moho rises from a depth of 70 km only about 50 km to the north to a depth of 50 km below the river at km 330, where it is interrupted. What we detect here is a very sharp limit at the Moho level to the south of the Lhasa block. Lithospheric perturbation in this region of former oceanic subduction and suture seemed likely. However, the sharpness of the Moho depth variation was unexpected. We should stress that seismograms on both sides of it were obtained at strictly the same offset from the shotpoint just on the suture, so that there can be no ambiguity of data reduction. If the mantle wedge on the northern side is indicative of the direction of thrusting during subduction, the exact coincidence of the Moho step with the edge of the Gangdise granitic belt, a major structural and mechanical boundary, points to a vertical contact of crustal segments. This is a strong argument for considering this boundary as related to post-suture strike-slip motion. This heterogeneity in the lithosphere may represent one of the major shear zones for the eastward expulsion of lithospheric segments in the continuing motion of India towards Asia⁶. This motion could have preserved and enhanced the sharpness and verticality of the shear zone and taken advantage of the east-west linearity of the original suture.

Three hundred kilometres further north the Lhasa block is thought to be limited by an earlier suture, the Bangong-Nujiang line. The northernmost part of our section crosses a branch of the related ophiolite belt. There is a striking

similarity between the northern 70 km of the Moho section and the topography beneath the Yarlung Zangbo suture. Here also we see a very sharp step of slightly less than 20 km in the depth of the Moho and the same polarity. The location of this step is coincident to within about 10 km with the geological boundary at the surface, so that the same aspect of probable verticality of contact between lithospheric segments is documented and the same considerations on post-suture strike-slip activity may hold.

Deep crustal features

For logistical reasons seismic recording stations were situated along the road through Lhasa, Yangbajain, Gulu and Nagqu, which is also a geologically well studied section. Because of the offset, however, seismic data sample the deep structure 100 km to the west, on the other side of the Nianqentangla range which interrupts the east-west structural trends. Thus, deep crustal features cannot be compared with geology in a simple way. In particular the extension to the west of the Damxung accident is not known and conversely the geological significance of the ophiolitic belt and of structural limits which are indicated on general maps and crossed by the seismic section, is not assessed. This central region appears to be the most complicated in the section. From km 330 to km 170 the average depth to the shallowest reflector of crust-mantle type apparently decreases from 70 to 50 km. However, at least four overlapping Moho segments can be identified, three of which are north-dipping, contrary to the general trend. From the Yarlung Zangbo, where it is at 50 km depth, the Moho dips to 70 km at km 300. At this point a further Moho segment is seen on top of it, dipping northwards from 55 km depth at km 300 to more than 75 km at km 230.

Near km 260, data to the south are from the Ang Ren shotpoint whereas to the north they are from Shi Lin Co. When approaching from the south the Moho dips from 60 to 70 km over 30 km distance. When approaching from the north the shallowest reflector is at 50 km, also dipping north. The energy, at this offset, of strong late arrivals suggests these can be attributed to a deeper crust-mantle velocity contrast. The depth and dip of this second level allow it to be tied in continuously through km 260 with the Moho defined from the other side and from another shotpoint. The geometry of these reflector segments could be described as the southward over-thrusting of three north-dipping lower crust layers on each other.

From km 230 to km 170 the depth to the Moho decreases from 50 to 40 km, although with local changes in dip. The succession of overlapping north-dipping reflectors followed in the south of the Lhasa block is interrupted by a new step when the shallowest level, 45 km, is reached at km 160. Contrary to findings in the south, one interpretation here is the northward overthrusting of a south-dipping deep crust segment. Alternatively, as second arrivals after those of the shallow reflector are not followed far to the south, a vertical step in the Moho without overlapping can be postulated.

In the region to the north (from km 120 to km 70), throughout the Bangge-Gulu granitic belt, the base of the crust is apparently about 50 km deep, the shallowest in the whole section. This zone is however tectonically very complicated, with further large ophiolite bodies. Variations in structure are also deduced from an additional east-west seismic profile perpendicular to the section presented here which may thus not be completely representative; furthermore, reflections deeper than this shallow Moho may be present. That such structure exists deeper can also be supposed from the significant signal level in later parts of the seismograms; it is likely that further crustal material is present beneath the shallowest mantle material at 50 km depth.

Tectonic interpretation

The considerable departure of the crust-mantle boundary from the horizontal along the section suggests that crustal thickening did not occur through mere underplating of several hundred kilometres of continuous Indian crust beneath Tibet, unless the

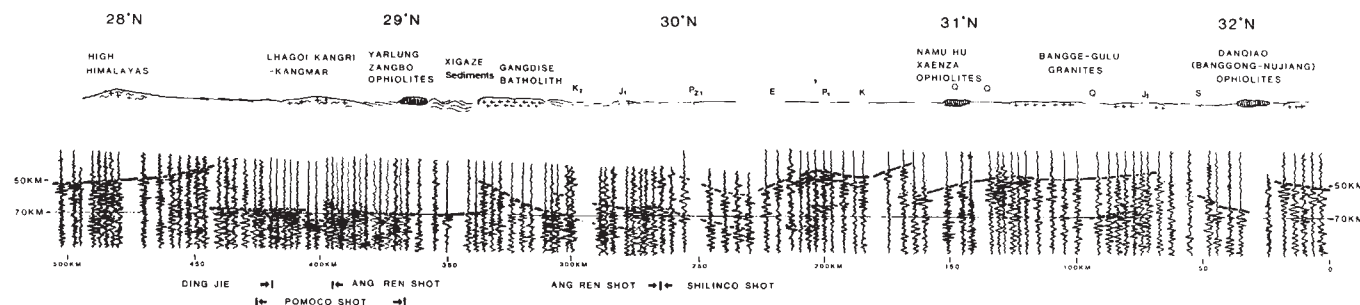


Fig. 2 Time-section of Moho reflections at constant offset of 250 km, that is, critical to the wide angle. This is a depth-section of the topography of the crust-mantle boundary along the line of Fig. 1 assuming negligible variation in average crustal velocity along the section. Approximate distances are counted along the north-south projection. Note the 20 km change in Moho depth across sutures (km 330, Yarlung Zangbo; km 30, Bangong-Nujiang; possibly km 160, Namu Hu ophiolites) thought to indicate later strike-slip motion on vertical block limits. Note also, for example, between km 180 and km 350, successive dipping and overlapping segments of crust-mantle reflectors indicating thickening of deep crustal levels as a result of the superposition of neighbouring pieces of lower crust along deep thrusts.

deep crust topography is due to more recent disruptions. We attribute the occurrence of vertical steps in the Moho, coinciding with major surface lineaments vertically above them (as documented for the two former suture zones bordering the Lhasa block), to such phenomena, mainly to horizontal strike-slip on major east-west lithospheric fractures due to the expulsion of Tibet to the east. The Moho step at km 160 could be interpreted in the same way unless the nearby ophiolite belt (west of Namu Hu) is an allochthonous unit or if prominent strike-slip features can be identified at the surface, as in the case of the Damxung accident on the other side of the Nainqentangla. The overlapping of dipping segments of the Moho noted at several places would suggest intracontinental thrusting of neighbouring crustal slices onto each other as a means of thickening the crust. No data on a possible subdivision of the thick crust of the Lhasa block are available as they were for the southern block towards the Himalayas.¹ It is worth noting however, that, as was the case there, the thickness of crustal units which are seen to interact at depth is less than 20 km. A similar picture of thickening of the lower crust by superposition of neighbouring lower crust units may thus be obtained. The complete thickening of

the crust would then also have needed superposition of neighbouring segments of upper crust layers along major thrusts. They could have the same polarity as the lower crust thrusts or the opposite one, as seems to be the case at the Himalayan border. As the part of the Lhasa block north of the Gangdise belt and west of Nainqentangla is not easy to reach, no geological study documenting surface expression of crustal thrusts is available with which to consider this hypothesis.

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1. Hirn, A. *et al.* *Nature* **307**, 23–25 (1984).
2. Hirn, A., Jobert, G., Wittlinger, G., Xu, Z. X. & Gao, E. Y. *Ann. Géophys.* (in the press).
3. Lépine, J. C., Hirn, A., Pandey, M. R. & Tater, J. M. *Ann. Géophys.* (in the press).
4. Hirn, A. & Sapin, M. *Ann. Géophys.* (in the press).
5. Oliver, J., Cook, F. & Brown, L. *J. geophys. Res.* **88**, 3329–3347 (1983).
6. Tapponnier, P., Peltzer, G., le Dain, A. Y., Armijo, R. & Cobbold, P. *Geology* **10**, 611–616 (1982).

Tectonic environment and geodynamic significance of the Neo-Cimmerian Donqiao ophiolite, Bangong-Nujiang suture zone, Tibet

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The Bangong-Nujiang suture zone is underlain by a discontinuous and roughly EW ophiolite belt. Around Donqiao–Gyanco, ophiolitic and metasedimentary Palaeozoic series form nappes thrust over Jurassic flysch series. All these series have undergone a late symmetrical folding accompanied by generally northward reverse faulting. Although strongly dismembered, a complete ophiolitic sequence comprising harzburgites with large podiform-type chromite deposits, wehrlites, cumulate gabbros, sheeted dolerite dykes, foliated garnet-bearing amphibolites and pillow lavas, has been found.

THE Tibetan plateau, which probably results from successive accretion of several continental blocks to the south of Eurasia, is cross-cut by two major EW suture zones^{1,2}. To the south, the Yarlung Zangbo suture zone is underlain by a nearly continuous ophiolite belt over several hundred kilometres. The latter separates the Indian plate to the south from the Lhasa block to the

north. In the Xigaze area, the ophiolites form a slice a few kilometres thick thrust to the south onto the Himalayan series. Although strongly dismembered, it shows a continuous ophiolite sequence, whose most peculiar feature is the occurrence of dolerite sills and dykes throughout the whole sequence from the basal fresh peridotites up to the Upper Albian–Lower

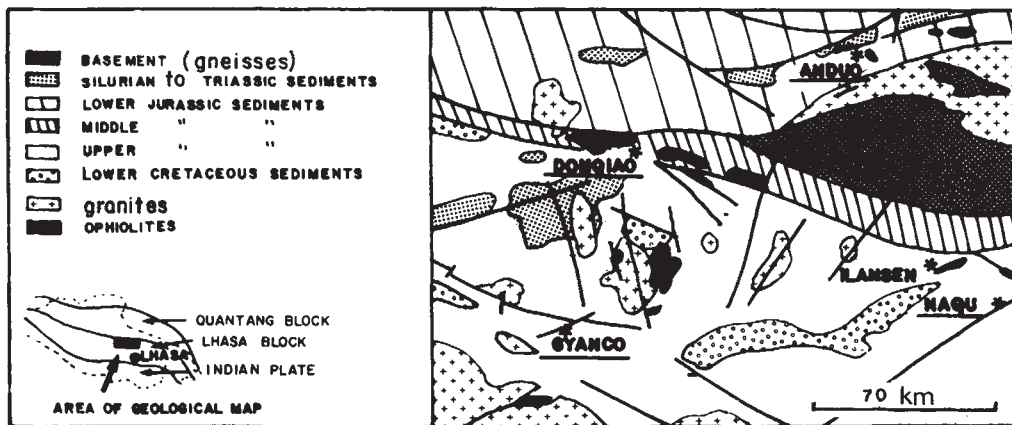
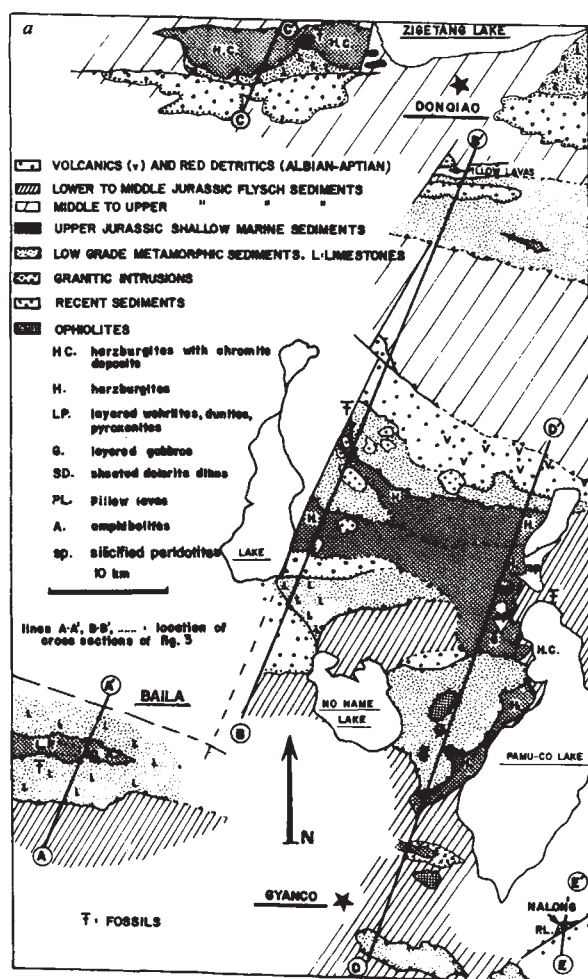


Fig. 1 The Bangong-Nujiang suture zone in the Anduo-Dongqiao-Gyanco-Gulu area (from the Chinese 1/1,500,000 scale geological map).



Cenomanian pillow lavas³⁻⁵. The ophiolite was probably obducted during the Eocene^{6,7}.

The Bangong-Nujiang suture zone⁸⁻¹¹ separates the Lhasa block to the south from the Quantang block to the north (Fig. 1). It is located approximately 300 km north of the Yarlung Zangbo suture zone and forms poorly defined EW belt several hundred kilometres long running from the north of Laddack to the west, to Nujiang valley (Salouen River) to the east, and is locally underlain by ophiolitic massifs. Very few data have been published on the tectonics of this suture zone⁸⁻¹¹.

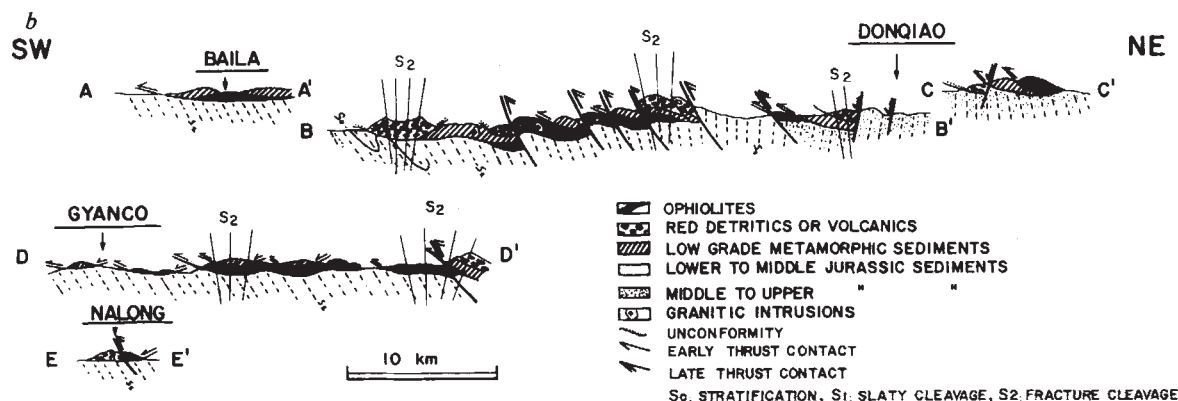
The observations presented here were obtained in the Dongqiao-Gyanco area, during the 1981 field work of the 3-yr French-Chinese joint project in Tibet. This area has been mapped at a scale 1:100,000 over a distance of nearly 100 km from north to south between Dongqiao and Gyanco, with an approximate EW width of 30 km.

A synthetic cross-section in that area shows, from bottom to top, a succession of Jurassic flysch, and ophiolitic and Palaeozoic metasedimentary series. They all stand in flat tectonic contact with each other and are overlaid in angular unconformity by Aptian-Albian red detritics and volcanics. The lithological and structural characteristics of the different series and their geodynamic significance are discussed.

Lithological and structural characteristics

The most representative outcrops of the Jurassic flysch series can be observed along the eastern side of the Pamu-Co Lake and immediately north of No-Name Lake (Fig. 2a). This series consists of rhythmic intercalations of dark shales, slates and yellow sandstones with scarce bio-detritic limestones forming lenses several metres thick. Fossils indicate a Middle Jurassic age (Aalenian) in the Gyanco area (*Gutnicella* (*Lucasella*) *cayeuxi*, *Haurania* sp. and *Nautilina* sp. in sample XSM 96).

Fig. 2 a, Lithological and structural map of the Dongqiao-Gyanco area. b, NNE-SSW sections through the Dongqiao-Gyanco area (location of sections on a; topography has not been respected).



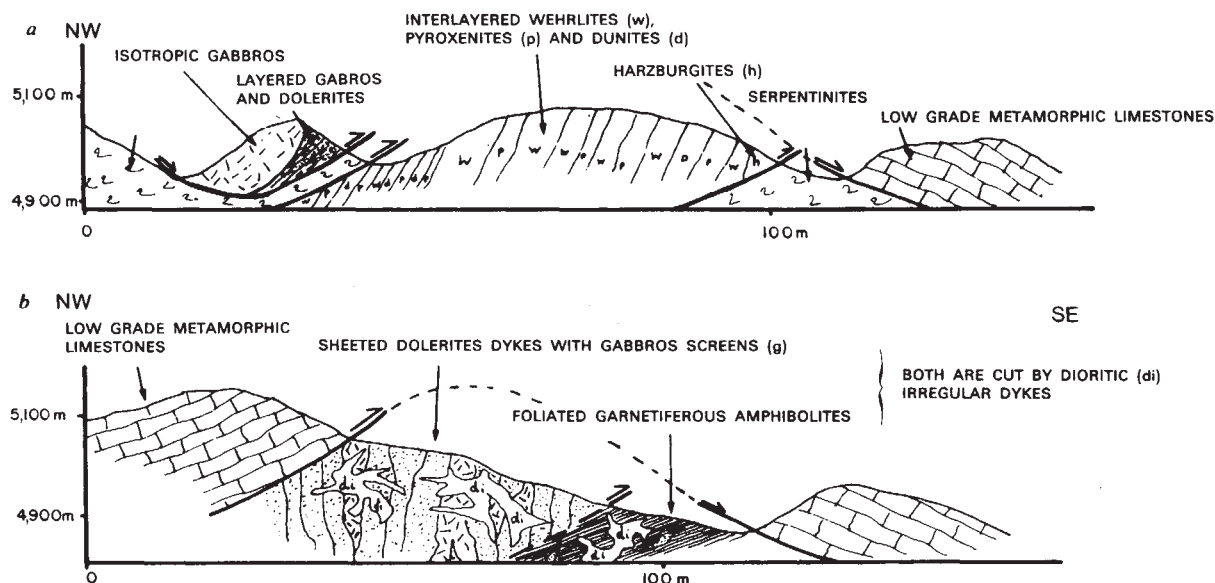


Fig. 3 NW-SE sections through the Baila massif. *a*, Wehrlites and gabbros section; *b*, sheeted dolerites and foliated amphibolite section.

and a Malm age around Donqiao (gastropods, *Sequania* sp.; G. Termier, personal communication).

This sedimentary series has undergone a low-grade metamorphism (sericite-chlorite) and shows N060 trending asymmetrical folds with subhorizontal axes and a generally north-dipping slaty cleavage S1 (~60°) parallel to their axial planes. In the Donqiao area, this cleavage is vertical or south-dipping (Fig. 2*b*). The F1 folds north of No-Name Lake seem to be overturned to the south. A subvertical late crenulation cleavage S2, related to large symmetrical folds, is locally superimposed on the previous structures.

The ophiolite series tectonically overlies the Jurassic sedimentary series everywhere, their basal contact being marked by low-temperature tectonic breccia, silicified peridotites or schistose serpentinites. Although the ophiolite sequence has been strongly dismembered and scattered, all the different parts of a typical ophiolite suite are found in the Donqiao-Gyanco area. They are:

(1) Residual harzburgites. These largely outcrop in the Donqiao massif and in minor amounts on the western side of Pamu-Co Lake, and in the Baila and Nalong massifs (Fig. 2*a*). Some serpentinites are also observed in the Ilansen area (Fig. 1) and about 20 km east of Amdo (Fig. 1). The harzburgites generally show porphyroclastic structures typical of low-stress high-temperature deformations^{12,13}. They are, however, serpentinitized and locally schistosed in shear zones related to EW subvertical late northward reverse faults. In some places the harzburgites are rich in dunite patches several metres in size and bear many podiform-type chromite deposits. These deposits, which are surrounded by irregular dunite walls, can reach several tens of metres in size and stand at a steep angle (40°) to the primary foliation plane. The chromite is massive with only a few local interstitial olivines transformed into serpentine. The foliation plane and lineation measured in the harzburgites show nearly constant orientations of about N060, NW70 and N055, NE17, respectively.

(2) Wehrlites, clinopyroxenites and dunites. These occur only in the Baila massif (Fig. 2*a*), approximately 30 km WNW of Gyanco where they outcrop as a window beneath Palaeozoic metasedimentary rocks (Fig. 2*a*). From south to north a section through this massif (Fig. 3*a*) shows (upwards) a few metres of harzburgites overlain by ~50 m of alternating bands (up to 5 m thick) of wehrlites and clinopyroxenites and ~30 m of more layered rocks composed of 10–30-cm thick layers of dunites, clinopyroxenites and wehrlites.

(3) Troctolites, layered and isotropic gabbros. These have been

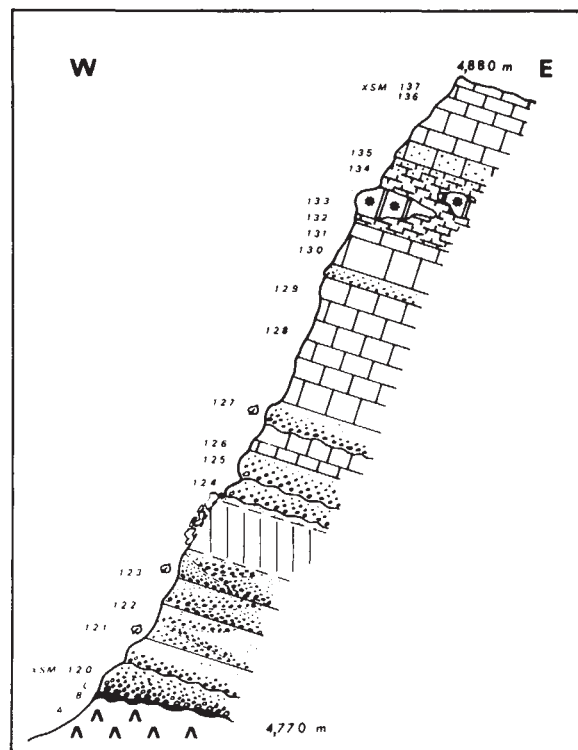


Fig. 4 The transgressive Zigetang formation. From bottom to top: A, serpentinitized harzburgites and dunites; B, red silicified encrustings; C, coarse-grained conglomerate with chromite pebbles. Sandstone member, XSM 120 to 127, thick-bedded terrigenous sandstones (cross-bedded laminations and plant remains); calcareous member, XSM 128 to 137, mainly well bedded calcareous mudstones, wackstones, subordinate terrigenous influx and patchreefs (XSM 133). Depositional environment: sub-aerial deltaic for XSM 120 to 127 to very shallow marine for XSM 128 to 137. Biostratigraphic content: XSM 122, grainstones with coral remains, *Cladocoropsis* sp., *Pseudochaetetes* sp., algae (Salemoporaceae), *Pycnoporidium lobatum* Y. and T. Foraminifers: *Kurnubia* sp. (or off. *Kurnubia*), *Pseudocyclammia* sp., *Nautiloculina* sp., XSM 133, boundstone with *Cladocoropsis* sp. and *Stromatoporidae*; XSM 137, mudstone, wackstone with *Dasycladacea*, *Salpingaporella annulata* Carozzi, *Trinocladus perplexus* Elliot.

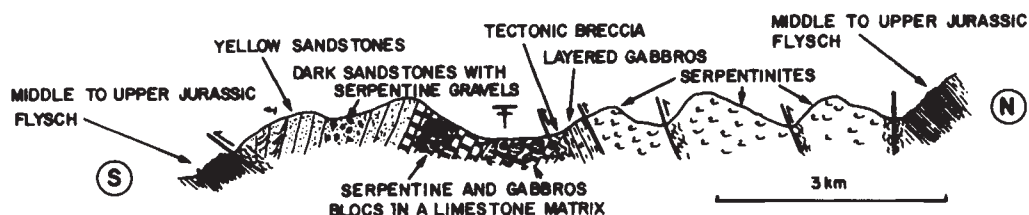


Fig. 5 NS synthetic section across the Ilansen olistostrome formation.

found on the western side of Pamu-Co Lake and in the Baila massif (Fig. 2a). Their total thickness does not exceed 50 m. In the Pamu-Co Lake area, troctolites form lenses several metres long and 5–10 m wide which are intercalated with olivine gabbros. They are overlain by a few tens of metres of slightly-layered plagioclase-rich gabbros. In the Baila massif, mafic rocks form a slice in tectonic contact over serpentinitized harzburgites and over the previously described wehrlites, clinopyroxenites and dunites (Fig. 3a). It consists of approximately 10 m of inter-layered fine-grained gabbros and dolerites overlain by 20–30 m of mainly brown amphibole-rich isotropic gabbros and dolerites in minor amounts.

(4) Sheeted dolerites and foliated garnet-bearing amphibolites. Approximately 50 m of sheeted dolerites overlie foliated garnet-bearing amphibolites a few tens of metres thick in the Baila massif area (Fig. 3b). The 1-m thick dolerite dykes are intrusive either in scarce isotropic gabbros or in older intrusive dolerite dykes. They generally show two chilled margins. The sheeted dolerites tectonically overlie foliated amphibolites. Both the sheeted dolerites and the foliated amphibolites are intruded by diffuse and interdigitated amphibole-bearing leucocratic diorite.

This late undeformed intrusive shows chilled margins with surrounding rocks.

(5) Lava flows and pillow lavas. These outcrop in the Nalong massif, ~20 km east of Gyanco (Fig. 2a), and also in the Anduo village (Fig. 1). In the Nalong massif, the volcanics form several tectonic slices limited by late reverse north-dipping faults, and are thrust to the south over the Aptian–Albian red continental series¹⁴. The volcanics, which are ~100 m thick, at their base (that is, to the south) comprise mainly massive lava flows several metres thick and, at the top, are formed of pillow lavas with a few hyaloclastite levels. The pillows (40–70 cm diameter in section) are generally variolitic. They display a general northward normal polarity. Lava flows and pillow lavas are cut by rare diabase dykes.

The allochthonous low-grade metamorphic sedimentary series is mostly composed of grey limestones, and of minor yellow-banded quartzites below dark shales. This series is observed in many places in the Donqiao–Gyanco area (Fig. 2a). This sedimentary series generally lies over the Jurassic flysch or the ophiolite series but can be locally overthrust by the ophiolite series, as in the Donqiao area (Fig. 2). We have found no specific

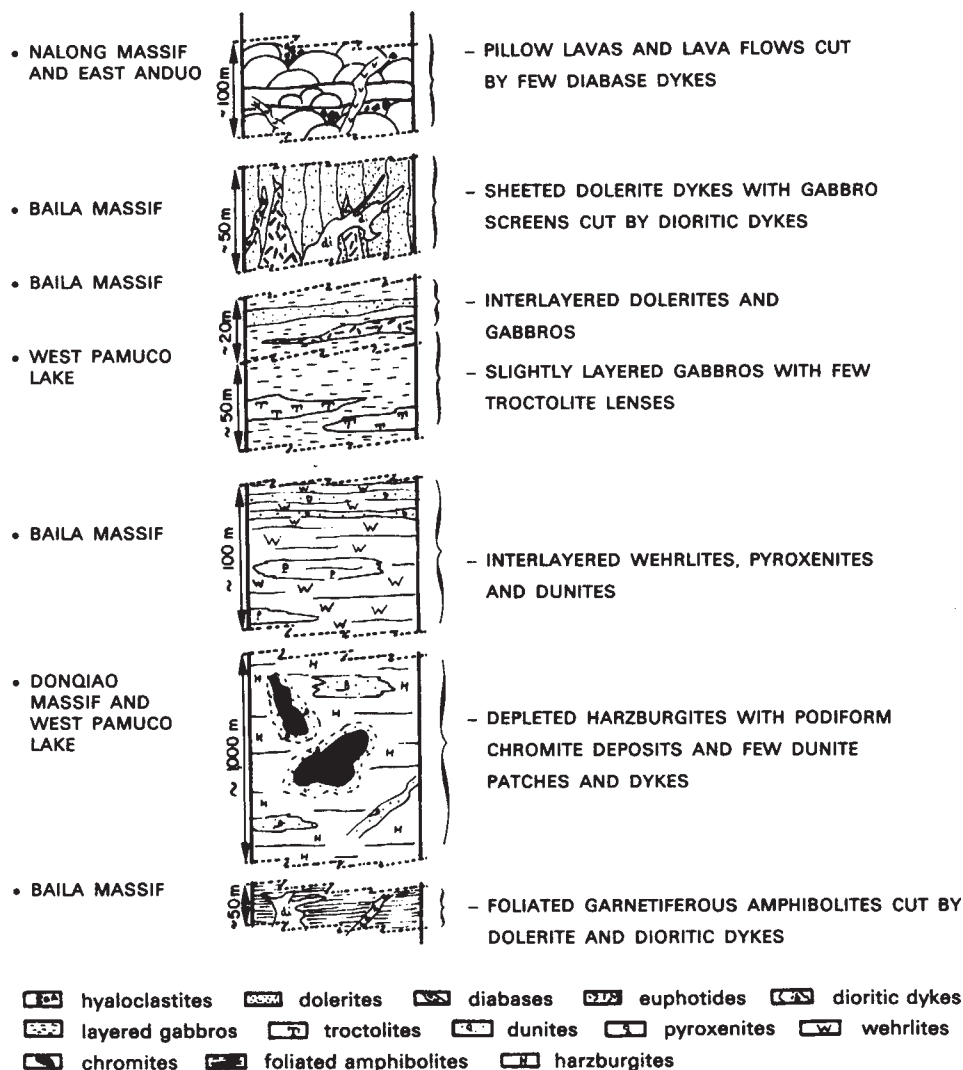


Fig. 6 Restored synthetic section through the Donqiao ophiolitic sequence.

fossils in this low-grade metamorphic formation, which is considered to be Upper Palaeozoic (C. F. Chang, personal communication). This series locally shows south-facing folds. It has been intruded by granites forming locally dykes oriented at about N 120, N 30.

Red detritics comprising conglomerates, red sandstones and shales largely outcrop in the Anduo–Donqiao–Gyanco–Gulu area (Fig. 1). Volcanics occur only north of Pamu–Co Lake where 5-m thick basaltic flows intercalated with thinner vesicular andesitic flows and a few ignimbritic flows lie unconformably above serpentinized peridotites (Fig. 2a). These volcanic series, some 10 m thick, are also covered in angular unconformity by red detritics beginning with coarse conglomeratic beds rich in pebbles of volcanics. Upwards, some thin pelite beds and rare ignimbrite flows a few centimetres thick are intercalated within the coarse conglomerate banks. These series, which are ~100 m thick, seem to be covered to the north by a thick yellow volcanic pile.

Biostratigraphical datations have given an Aptian–Albian age for the red sandstones and pelites which outcrop east of Gyanco¹⁴. The volcanics or red detritics overlie in unconformity the Jurassic flysch series as well as the ophiolitic or Palaeozoic metasedimentary series described above. The volcanics and red detritics are not metamorphic but, in addition to the underlying series, have undergone a symmetrical folding with N 140 axes, accompanied by a fan-shaped spaced cleavage.

North of Donqiao, the chromite-bearing peridotites (Fig. 2a) are covered by a transgressive detritic shallow marine formation called the Zigetang formation¹⁵. It begins with coarse-grained conglomeratic levels containing chromite pebbles transgressing red silicified peridotites (Fig. 4). At the top the conglomerate beds are interbedded with thick terrigenous sandstone beds. This sub-aerial deltaic 40-m thick unit is covered by very shallow marine sediments mainly comprising well bedded calcareous mudstones, wackstones, subordinate terrigene influx and some patchreefs. The thickness of this strongly eroded formation does not exceed 130 m. The numerous foraminifers and algae we have found (see Fig. 4 legend) indicate an uppermost Jurassic–lowermost Cretaceous age.

In the Ilansen area (Fig. 1) the ophiolitic series constitute an olistostrome formation southward thrust over an Upper Jurassic flysch series. From north to south a section across this massif (Fig. 5) shows (downwards) the stratigraphical succession of yellow sandstones displaying abundant cross-bedded figures indicating a normal polarity to the south, serpentine-bearing sandstones and grey limestones locally rich in angular layered gabbro blocks of various sizes. The contact between the limestones and the peridotites to the south has not been observed. The grey limestones in which the gabbro blocks are cemented are rich in corals and gastropods, indicating a probable Upper Jurassic age.

The Donqiao ophiolite

A typical ophiolitic sequence, though strongly reduced in thickness (the cumulated thickness is 1,400 m), has been found in the Donqiao–Gyanco area (Fig. 6). This ophiolite is very similar to those previously described elsewhere¹⁶. It is composed of a mantle part comprising harzburgites which clearly represent residual rocks, wehrlites, pyroxenites and dunites which can be either cumulates or impregnation rocks, and of a crustal part composed of cumulate-layered gabbros, sheeted dolerites and volcanics. The foliated garnet-bearing amphibolites, which lie

beneath the sheeted dolerites, could represent remnants of a metamorphic sole, as is seen beneath many ophiolites¹⁷. The occurrence of large podiform-type chromite deposits is the only peculiarity of this ophiolite sequence. The first geochemical data analysis performed on this ophiolite indicates that it could have been formed in a small ocean in a subduction zone environment and is distinct from the Kigaze ophiolite to the south¹⁸.

Tectonics and geodynamics

Several successive tectonic events can be defined in the different units:

- (1) The first tectonic event, D1, has led to folding of the Jurassic flysch series in which south-facing folds accompanied by axial-plane slaty cleavage can be locally observed. This tectonic event has been followed by a large thrusting tectonic event D1-2 corresponding to the southward emplacement of the ophiolites and of the Palaeozoic metasedimentary rocks onto the Jurassic flysch series. Thrusting was from north to south as demonstrated by the NS direction of stretching lineations in limestones beneath the ophiolite series and by subsequent shear structures.
- (2) A second folding event, D2, inducing the formation of large symmetrical folds accompanied by vertical fracture cleavage postdates the deposition of the Aptian–Albian red detritics and associated volcanics and deforms all the basal sedimentary or thrust contact planes. It is followed by large reverse faultings producing thrusting of the Upper Jurassic flysch and of the ophiolites over the Lower Jurassic flysch and red detritics, respectively.

The obduction of the Donqiao–Gyanco ophiolite occurred at the Upper Jurassic, as the ophiolite is transgressively covered by uppermost Jurassic–lowermost Cretaceous sub-aerial to shallow marine sediments.

It is therefore likely that the D1 tectonic event observed in the Middle to Upper Jurassic flysch series is due to the obduction of the ophiolite. The obduction probably occurs from north to south as indicated by both the vergence of folds in Jurassic flysch series and the stretching direction and sense determined at the base of the nappes. This obduction can be related to a north-dipping subduction zone which should have been located northwards and could have resulted from the uppermost Jurassic–Lower Cretaceous collision between the Lhasa and the Qiantang blocks.

The D2 folding and associated reverse faulting events occurred in the same time span as the folding of the Takana formation to the south (upper part of the Lower Cretaceous) which is probably related to the Eocene, India–Eurasia collision^{2,6,7}.

The Donqiao ophiolite yields new information on the Bangong–Nujiang suture zone. A quite similar suture zone has been described west of Tibet in the Farah Rud, Afghanistan^{19,20} and to the east in the Sittang Valley Myitkyina zone in Burma^{21,22}, where the obduction of the ophiolitic series occurred at the same time (Upper Jurassic to lowermost Cretaceous) during a wide Neo-Cimmerian tectonic crisis along the South Eurasian margin.

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1. Chang, C. F. & Cheng, H. I. *Scientia sin.* **16**, 257–265 (1973).
2. Tapponnier, P. et al. *Nature* **294**, 403–409 (1981).
3. Nicolas, A. et al. *Nature* **294**, 414–417 (1981).
4. Marcoux, J. et al. *Ophiolite* **6**, Abstr. (1981).
5. Girardeau, J., Nicolas, A., Marcoux, J. & Zhao, Y. *Eclog. Helv.* (submitted).
6. Dewey, J. P. & Bird, J. M., *J. geophys. Res.* **75**, 2625–2647 (1970).
7. Molnar, P. & Tapponnier, P. *Sciences* **189**, 419–426 (1975).
8. Bally, A. W. et al. *U. S. Geological Survey Open File Report*, 80–501 (1980).
9. Chang, C. F., Zheng, X. L. & Pan Y. P. *Chinese Acad. geol. Sci.* **2**, 1–17 (1977).
10. Li, C. Y. et al. *Chinese Acad. geol. Sci.* (1979).

11. Girardeau, J. et al. *Réun. Ann. Sci. Terre. Abstr.* **12**, 179 (1982).
12. Mercier, J. C. C. & Nicolas, A. *J. Petrol.* **16**, 454–487 (1975).
13. Nicolas, A., Boudier, F. & Bouchez, J. L. *Am. J. Sci.* **279A**, 543–576 (1980).
14. Jaeger, J. J. et al. *EOS* **63**, 1093 Abstr. (1982).
15. Chang, C. F. & Pan, Y. S. *Proc. Symp. Qinghai, Xizang, Beijing* (Acad. Sinica, 1981).
16. Coleman, R. G. *Springer Verlag Minerals and Rocks* **12**, (1977).
17. Williams, H. & Smyth, W. R. *Am. J. Petrol.* **279**, 594–621 (1973).
18. Gopel, C. et al. *EOS*, **63**, 1095, Abstr. (1982).
19. Blaise, J., Bordet, P., Carbone, J. P. & Montenat, C. *Bull. Soc. Géol. Fr.* **7**, 795–798 (1978).
20. Bassoullet, J. P. et al. *Coll. C5 Memoire BRGM* **115**, 180–198 (1980).
21. Mitchell, A. H. F. *J. geol. Soc. Lond.* **138**, 109–122 (1981).
22. Sengor, A. M. C. *Proc. Symp. Qinghai, Xizang, Beijing*, Vol. 1 (Acad. Sinica, 1981).

High heat flow in southern Tibet

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Heat flow measurements have been attempted in two freshwater lakes at altitudes of 4.5 and 5.0 km, south of the Yarlung–Zangbo suture zone in southern Tibet. Probe penetration in the lake sediments was deep enough in the case of eight measurements (5.5–7.2 m) to give reliable temperature gradients. Corrections for seasonal temperature variations, topographic and refraction effects have been applied to the data. In a north–south profile trending perpendicular to the Yarlung–Zangbo suture zone, heat flow is approximately constant at 146 mW m^{-2} over a distance of 30 km and drops to a value of 91 mW m^{-2} in $<25 \text{ km}$. The high heat flow and the sharp spatial variation both suggest the existence of a heat anomaly located at relatively shallow depths (no greater than 25 km) in the Tibetan crust, probably due to the recent emplacement of plutonic bodies.

TIBET is a massive plateau with a vast areal extent ($2.5 \times 10^6 \text{ km}^2$) at an unusually high elevation (5 km above sea level). It holds a privileged geodynamic position north of the India–Asia collision zone between the Himalaya and Kun Lun mountain ranges. The origin of this remarkable broad regional uplift is still unresolved. Somehow, it must be related to the overly thick (70-km) crust determined by different seismic studies^{1,22}. Any model for the plateau uplift must use as a constraint the thermal regime at depth. So far, educated guesses have only been made about a representative geotherm when trying to model the tectonics of Tibet² or when trying to recover the uplift rate from fission track analyses in Himalayan rocks³. The Tibetan plateau is characterized by strong geothermal activity with numerous boiling springs, hot springs and even geysers scattered over most of its area⁴. Because the rheology of rocks depends strongly on temperature, the thermal structure of Tibet is a most important parameter for understanding the processes active in a continent–continent collision zone. Some predictions have been made. Wang and others² assumed a heat flow value of 84 mW m^{-2} on the basis of poorly constrained correlations between heat flow and age in geological provinces⁵. The existence of a thick crust enriched in radioactive elements would also yield a high heat flow. The lack of conductive heat flow measurements leaves the question unsettled. During the last field programme of the French–Chinese cooperation for the study of Tibet and the northern Himalayas which was started in 1980, it was decided to carry out heat flow measurements using oceanographic techniques in the numerous lakes which dot Tibet. The measurements reported here are the first known conductive heat flow measurements in Tibet.

Measurements

Many heat flow measurements have been made in lakes using the oceanic probe technique⁶. They have the obvious advantage that the cost and time required are much less than those needed for boreholes, tunnels or mines. Unfortunately, they are associated with many difficulties. For example, the recent and fast erosion of a lake depression leads to an artificial increase of temperature gradients⁷. These difficulties have been encountered in the Alps where deep lake heat flow data appear systematically higher than tunnel or borehole data^{7,8}. There are fewer problems in small and shallow lakes which have been shown to yield results comparable to those from deep boreholes⁹. The ideal lake is probably not too deep, so that the transient erosion effects mentioned above are small, and not too shallow, so that thermal conditions in the lake are rather stable. In Tibet, our main problem has been to find such lakes as well as a suitable vessel from which to conduct the measurements.

We used a pontoon, which could be assembled and disassembled in a few hours, $10 \times 12 \text{ m}$, about 1 m high on the water and powered by three outboard motors. A derrick had been erected in the middle and a gasoline-powered winch permitted the instruments to be lowered on a standard steel cable.

We used seven equally spaced (60 cm) thermistors mounted on outriggers tied either to the 4.5-m long barrel of a standard Kullenberg piston corer or to a thin (48-mm diameter) 6-m long metal rod used in quarry drilling operations. Both the corer barrel or the metal rod were clamped to a Kullenberg coring head with $\sim 250 \text{ kg}$ weight. A trigger weight enabled the barrel

Table 1 Heat flow data

Station no.	Lat.	Long.	Water depth (m)	Penetration depth (m)	G_1 ($^{\circ}\text{C km}^{-1}$)	T_1 ($^{\circ}\text{C}$)	G_2 ($^{\circ}\text{C km}^{-1}$)	Steady-state correction			Heat flow (mW m^{-2})
								Topography (%)	Refraction (%)	Warm rim (%)	
6	29°10'15"	90°37'00"	40	3.9	/	5.07	185	-13.3	+2.6	-7.2	127
10	29°08'00"	90°41'30"	40	3.9	/	5.08	218	-7.1	+0.8	-3.0	166
14	28°58'45"	90°45'00"	44	6.7	192	5.11	176	-4.3	+1.6	-3.2	138
15	28°51'30"	90°37'30"	33	6.3	294	5.10	299	-1.6	+0.3	-1.3	242
16	28°50'45"	90°36'45"	31	7.0	206	5.11	188	-2.0	+0.4	-1.4	152
17	28°34'00"	90°26'45"	53	7.0	99	4.78	101	/	/	/	88
18	28°33'30"	90°24'45"	57	7.2	101	4.74	103	-1.8	+1.8	-0.3	90
19	28°33'45"	90°28'30"	60	6.9	116	4.88	101	-0.3	+1.0	-0.2	88
20	28°34'00"	90°28'45"	65	7.2	118	4.80	114	-0.2	+1.1	-0.2	100
21	28°34'45"	90°28'15"	60	7.2	131	4.85	101	-0.3	+0.8	-0.4	88

The average heat flow for Yang-Zho-Yung lake (stations 6, 10, 14–16) = $146 \pm 17 \text{ mW m}^{-2}$ (excluding station 15). Average heat flow for Pu-Mu-Yung lake (stations 17–21) = $91 \pm 5 \text{ mW m}^{-2}$. G_1 is the uncorrected temperature gradient which can be estimated from the deepest data points. G_2 is the temperature gradient after correcting for seasonal bottom-lake temperature fluctuations. T_1 is the average bottom-lake temperature, obtained after extrapolating the corrected temperatures.

or rod to free fall through the last 2 m of the water column before penetrating the sediments.

The temperature recorder for the seven sediments and one water temperatures was housed in a basket fastened to the coring head. We used an AANDERAA recorder modified for oceanic heat flow work at the Centre Oceanologique de Bretagne, France. Temperatures were recorded internally on magnetic tape which could be read later in our base camp Lang-Ka-Zhe (Fig. 1).

Water depth was monitored by a Furuno FE-400C echosounder operating at 50 kHz and good to 480 m. We lacked a 3.5-kHz seismic system so that the sediment thickness was assumed from previous drilling by a Chinese party in one of the lakes (Yang-Zho-Yung lake). Navigation was by means of triangulation on key-features of the shore using large-scale topographic maps (1:100,000) and a hydrographic circle good to 1°.

Conductivity measurements were made on cores using the standard needle-probe method¹¹.

The two lakes where heat flow work was feasible during our field trip are located south of the Yarlung-Zangbo suture zone (Fig. 1). The northern lake, Yang-Zho-Yung, lies 4,400 m south of a prominent range. It is very tortuous in plan view (Fig. 1) and clearly represents a blocked river valley which used to drain into the Zangbo river and which probably got cut off through tilting in the active extensional tectonic zone striking roughly north-south between Yadong and Gulu¹² (Fig. 1). On the basis of a preliminary description of one core from the deepest part of the lake (core K1), the sediments consist mainly of light-grey, carbonate-rich muds including many layers with grassy mats and seem to have sedimented quietly (K. Kelts, personal communication).

The southern lake, Pu-Mu-Yung, lies at 5,000 m altitude just to the north of the Himalayan mountain range (Fig. 1). Some glaciers feed into the lake. One core (core K4) shows that the sediments are siltier with less admixture of carbonate and fewer diatoms. The core material is being studied for mineralogy, ¹⁴C dating, palynology, organic content and ²¹⁰Pb.

The temperature measurements were conducted by selecting a site away from the shore, lowering the probe 10 or 5 m off the bottom for a few minutes, free-falling the probe into the sediments and waiting for 17 and up to 70 min in the sediments. The thermistors were off scale in the bottom water so that possible instrumental bias between the probes could not be removed.

Results

Four cores about 5 m long were taken before or during the temperature measurements. To check on possible conductivity changes between stations, outrigger sampling was undertaken at 12 stations. A total of 124 conductivity measurements including 24 repeated runs were made within 12 h after samples were retrieved from the lake bottoms.

The results show that thermal conductivity is rather uniform both horizontally and vertically (Fig. 2). There is good agreement between measurements on core and outrigger samples, although a subtle tendency can be detected showing a slight underestimation of conductivity in the outrigger samples, which can be attributed to a slightly different water content. There is no systematic variation of thermal conductivity with depth, except for the lowermost part of the cores (40 cm) where a dramatic increase of *k* values occurs, presumably due to compaction at the end of penetration (Fig. 2). These high values were omitted from the data set. We therefore used simple average values of 2.00 ± 0.16 (8%) and 2.09 ± 0.20 (9%), in units of $10^{-3} \text{ cal cm}^{-1} \text{ s}^{-1} \text{ }^{\circ}\text{C}^{-1}$, for Yang-Zho-Yung and Pu-Mu-Yung lakes respectively.

According to the diffusivity-conductivity relation reported by Von Herzen and Maxwell¹¹, the thermal diffusivity of these sediments should be $\sim 2.5 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$. This value will be used throughout this study.

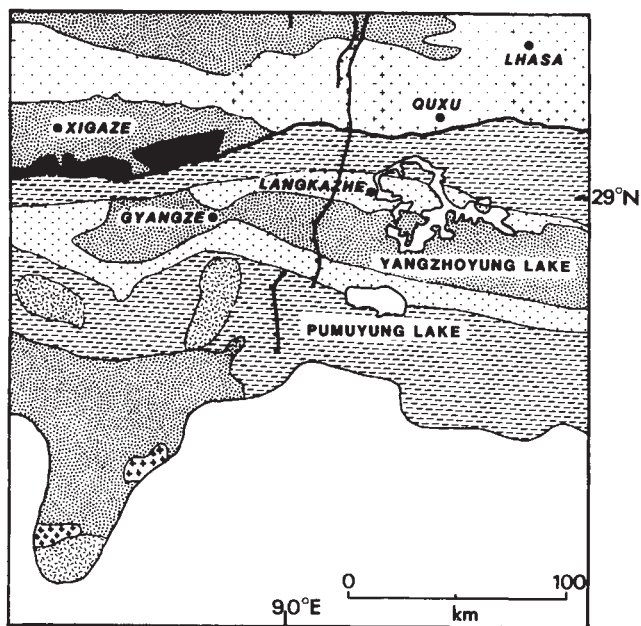


Fig. 1 Schematic geological map of Tibet in the vicinity of Lhasa showing the location of the two lakes where heat flow measurements have been made (modified from ref. 12).

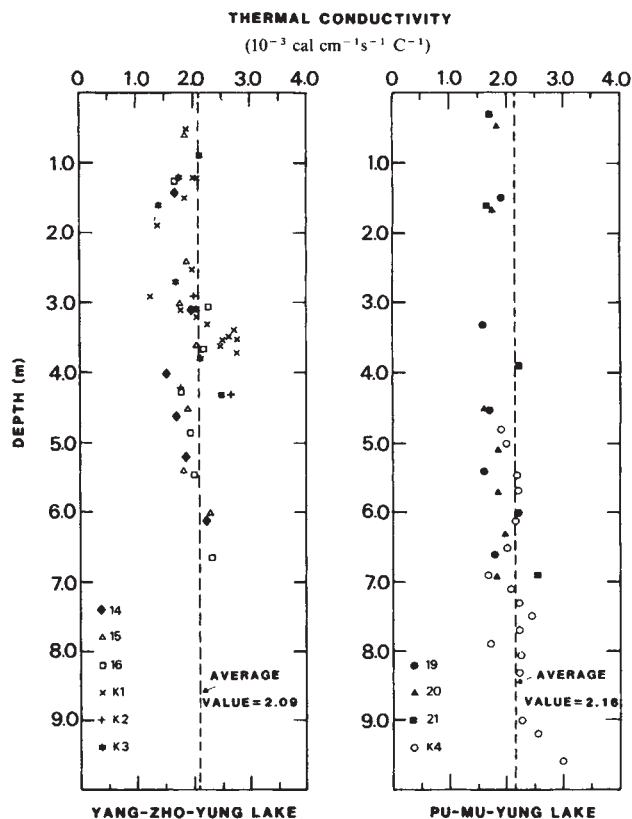


Fig. 2 Thermal conductivity of sediments from the two Tibetan lakes. The average value is that for all measurements.

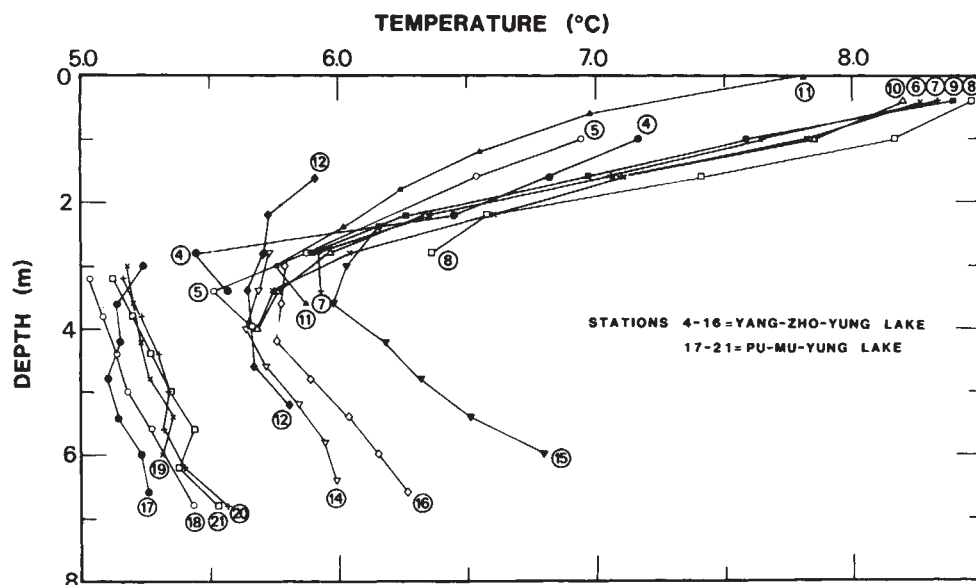


Fig. 3 Temperature versus depth plots for all the heat flow stations of this study.

Seventeen temperature stations were achieved with penetration depths ranging from 2.7 to 7.2 m (Table 1). For most of the stations, the duration of stationing was sufficiently long (>20 min) for equilibrium temperatures to be obtained. All the available measurements can be seen in Fig. 3. Note the good consistency of the data within each lake, and the marked difference between the two lakes. The data are believed to be reliable except for stations 4 and 5 where the measurement duration was too short to yield equilibrium conditions. In stations 11 and 17, the estimates of penetration depths seem to be wrong. Above a depth of 4–5 m, temperatures are obviously affected dramatically by seasonal climatic variations.

We now turn to the various corrections which must be applied to these raw data.

The correction for lake-bottom water temperature fluctuations requires the knowledge of the sediment–water interface temperature variations. In the remote highlands of the Tibetan plateau, none of these data are available or easy to obtain. However, climatic records are available over a 20-yr period from the Lang-Ka-Zhe meteorological station close to our lakes (Fig. 1). Figure 4 shows the evolution of the yearly mean temperature over this length of time, as well as the monthly mean air and ground temperatures. The data can be fitted by a simple cosine function with a period of 1 yr (Fig. 4). The lakes do not freeze in the winter season. The external forcing due to weather conditions is regular and it is likely that the lake water temperature varies with the same 1 yr period. In any case, the 1 yr cycle with 8 °C amplitude must be the dominant component of temperature fluctuations.

Fluctuations with shorter periods are possible due to transient water transport phenomena in the lakes, but would be of smaller magnitude and of much smaller depth penetration. For an amplitude T_0 and a pulsation ω , the resulting amplitude of fluctuations at depth z is (ref. 13, p. 63)

$$T_0 \exp\{-(\omega/2\kappa)^{1/2}z\} \quad (1)$$

Considering the high gradients observed, such fluctuations must be smaller than 0.02 °C in amplitude to be considered as negligible. For the main 1 yr cycle, we found that a good estimate for T_0 was 4 °C. From equation (1), this perturbation has no measurable effect at depths >8.4 m. Shorter period fluctuations must be of smaller amplitudes. Taking for example, a 1 °C amplitude and a 6-month period, we find that the effects are significant down to depths of 4 m only.

As a consequence, we assumed that the major correction to be applied is for a 1 yr cycle, with an amplitude smaller than that of the air temperature (T_0) and a time-delay (ϕ) due to the lake response. The formula used for the temperature fluctuations at depth z is:

$$T_0 \exp\{-\alpha z\} \cos\{(\Pi/6)t - (7.5/6)\Pi - \phi - \alpha z\} \quad (2)$$

where α is equal to $\{\omega/2\kappa\}^{1/2}$, ω is the pulsation ($\Pi/6$) and t is time (in months). The correction was applied to all stations on a trial basis, varying both the amplitude and the delay, until optimum linear profiles were obtained. Parameters used in establishing the best correction were: amplitudes of 3.2–4.0 °C for both lakes, and small delays of 1.3–1.7 months which are not unexpected since the lakes are rather shallow. The extrapolated sediment–water interface temperatures were checked to be uniform in all cases (Table 1). In each lake, a single type of correction was found to correct most of the stations. The corrected gradients are in good agreement with each other, except for station 15 which yields a very high value. We attribute this anomaly to local refraction and topographic effects because this station is close to a small island in Yang-Zho-Yung lake (Fig. 5). No correction could be applied with reasonable success to several stations with shallow penetrations in Yang-Zho-Yung lake. This is probably due to temperature fluctuations with periods smaller than 1 yr, over which we have no control but which have negligible effects on the deeper data points. These stations do not constitute a heavy loss because they are clustered near stations 6 and 10 where reasonable corrections could be applied. A more detailed discussion of these corrections will be reported elsewhere.

Steady-state corrections are made up of corrections for local topography, thermal refraction at the sediment–basement boundary, and the warm-rim effect of the lake.

These effects were estimated numerically using a finite-element computer program. Calculations were done mostly at the Institute of Geology, Academia Sinica, Beijing and some on a different program in Paris. We took seven profiles between 14 and 30 km long, roughly perpendicular to local topographic features, which covered all heat flow stations. Most of these corrections are of small magnitude (<10%) (Table 1).

Transient corrections involve long-term climatic changes, sedimentation rates and uplift and erosion. Using available climatic models for Tibet¹⁴ and the preliminary observation that the lakes have sedimented quietly, the first two corrections are found to be small (~1.7–3.5% and 1.0–2.6% for climatic and sedimentation effects respectively). The correction for uplift and erosion is more difficult to evaluate because we have no good estimates for erosion and uplift rates. We now discuss the implications of these measurements, with due consideration for the remaining uncertainties.

Discussion

Figure 5 summarizes the heat flow values obtained in this study after all corrections excluding those for uplift and erosion. Note the good consistency of the values within each lake. Given the magnitude of measurement errors which is certainly larger than

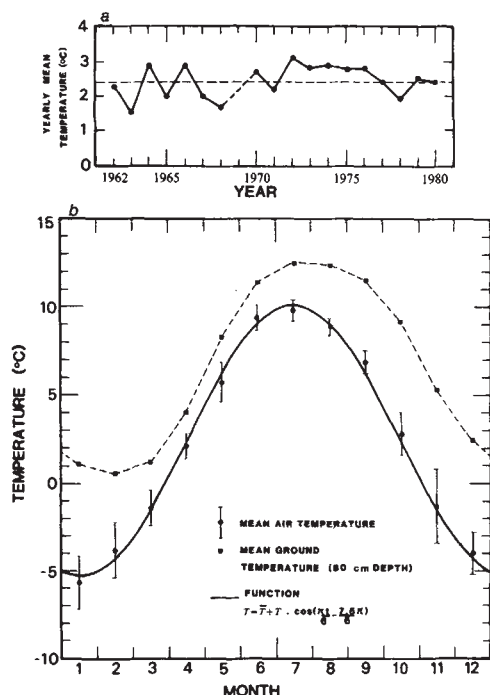


Fig. 4 a, Evolution of the yearly mean temperature at the Lang-Ka-Zhe meteorological station. b, Evolution of the air and ground temperature (at 80 cm depth) over a period of 1 yr. The air temperature data is made of average monthly values over 20 yr (bars represent ± 1 s.d.). Also shown is the simplest cosine function which fits these data.

10% (a representative value for the conventional oceanographic technique), the data do not provide any evidence for a significant variation of heat flow within Yang-Zho-Yung Lake. Our measurements show that heat flow rises sharply from 91 to 146 mW m^{-2} in $\approx 25 \text{ km}$, and remains approximately constant over a distance of 30 km towards the Yarlung-Zangbo suture zone (Fig. 6). There are *a priori* three possible explanations for such high heat flow values: high heat production, an anomalous heat source (magma), or an anomalous mantle heat flow. A detailed interpretation of the data requires a proper understanding of: (1) uplift and erosion; (2) the regional thermal structure and its evolution with time; and (3) the age of the hypothetical intrusion or melting event. More extensive thermal modelling will be reported elsewhere.

The uplift problem is probably the most crucial one. Several authors have argued on palaeontological evidence and fission track studies³ that the uplift is recent (2 Myr) and fast (0.2 cm yr^{-1}). Such fast uplift rates could increase the heat flow values by as much as 60%. However, some of these estimates depend on an assumed thermal regime and the reality of such a dramatic pulse remains questionable. The India-Asia collision has been occurring for $\sim 45 \text{ Myr}$ (ref. 15) and there is no obvious reason why a major change in tectonic regime occurred 2 Myr ago. Typical epeirogenic uplift rates in active regions are of the order of 0.2 mm yr^{-1} (ref. 16), which would spread out in time the plateau uplifting, making it more compatible with the overall evolution of the region. In such a case, the uplift effect would be much smaller, around 20%. However, these corrections were estimated in the usual fashion (ref. 13, p. 338) and, in the very transient thermal state of Tibet, are of little interpretative value.

Erosion effects must also be taken into consideration. In the absence of any information concerning the shaping of the present terrain morphology, it is impossible to estimate the effects of local and recent erosion, such as those discussed by England⁷. In the plateau environment, they are probably small because local topography is very subdued, as witnessed by the small magnitude of the topographic correction (Table 1). Relief is more pronounced near sites 6 and 10 in Yang-Zho-Yung Lake. However, these stations yield values which are comparable to

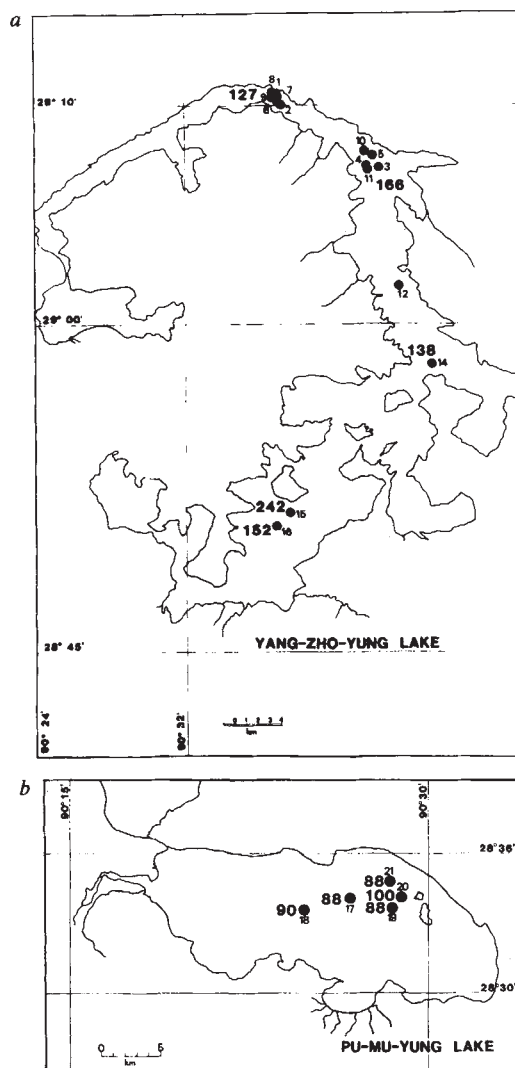


Fig. 5 a, Heat flow stations (small numbers) and values (large numbers) in Yang-Zho-Yung lake. Heat flow values are in mW m^{-2} (not corrected for uplift and erosion, see text). b, As a for Pu-Mu-Yung lake.

those from stations 14 and 16, 20 km away to the South where local topography is negligible ($< 70 \text{ m}$). We believe that the difference between the heat flow values for the two lakes is real. As will be stated later, electromagnetic measurements yield evidence for a discontinuity at the same location. The regional erosion of the Tibetan highlands is more of a problem. It represents an additional increase of heat flow, as it corresponds to a vertical advection of hot material (see, for example, ref. 17).

Note that in the extreme conditions prevailing here, the usual procedure for 'correction' is not valid. It is designed to account for transient effects which are significant at shallow depths only in otherwise steady-state conditions and to allow the calculation of representative geotherms. In the case of Tibet, we must take into account the full transient thermal evolution of the region, including crustal shortening and the resulting stretching of geotherms¹⁸ as well as regional cooling due to the underthrusting of cold continental lithosphere.

At any rate, such regional phenomena must affect all heat flow values and cannot account for the sharp spatial variation which is observed. Even if the maximum correction of 60% is applied, the Yang-Zho-Yung values would still be high by continental standards: at 58 mW m^{-2} they would clearly stand above 'equilibrium' regions where the mean heat flow is 46 mW m^{-2} (ref. 19). This difference is not due to anomalously high heat production. There are few radioactivity determinations on Tibetan rocks. The available data from basement material

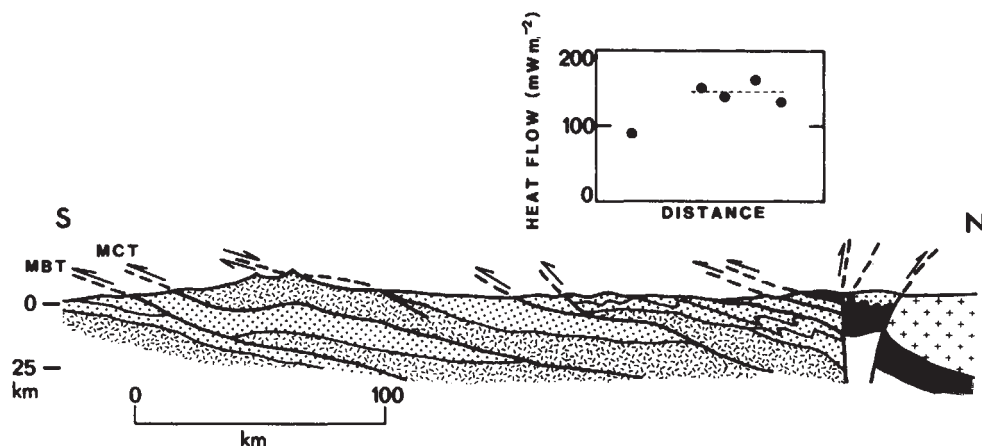


Fig. 6 Schematic cross-section perpendicular to the Yarlung-Zangbo suture zone (from ref. 15). Crosses and dots indicate granitic and sedimentary formations. The stippled and grey patterns are for basement and ophiolite rocks. The heat flow values are shown at their respective locations.

which outcrops to the West of our lakes (Fig. 1) indicate a value of $2.7 \mu\text{W m}^{-3}$ for the heat production rate²⁰. This value is normal for old continental formations, and, according to empirical heat flow/heat production relations, corresponds to heat flow values around 45 mW m^{-2} (see, for example, ref. 19).

One explanation for the high heat flow values would be that because the crust is anomalously thick, there is a larger amount of radioactive elements beneath the plateau. Considering an average heat production of $0.7 \mu\text{W m}^{-3}$ for continental crust material²¹, it would take a thickness of 140 km to yield a radiogenic contribution of 100 mW m^{-2} to the surface heat flow (that is corresponding to an observed average heat flow of 125 mW m^{-2}). Furthermore, we would also have to explain why heat flow increases so rapidly between the two lakes. Similarly, an anomalous mantle heat flow is not a suitable hypothesis because it should have a regional effect. Even if it was localized in a narrow zone below the lithosphere, it could not create a sharp horizontal variation because of the masking effect of the plate.

The most likely explanation is therefore that there is a source of heat in the crust which terminates abruptly somewhere between the two lakes. Such a discontinuity at this particular spot was also found from magneto-telluric soundings carried out in the same area²¹. This anomalous source of heat is most probably a hot intrusive body. To model the associated thermal effects would require some knowledge about the time of emplacement or melting: a recent event must be shallow to be detectable by surface heat flow measurements. The conductive time scale is $\tau = d^2/4\kappa$, where d is the depth to the anomaly and κ thermal diffusivity. Because the intrusive or melting event must somehow be associated with the underthrusting of India beneath Asia, it must be younger than 45 Myr^{15,22,23} and must therefore be shallower than ~70 km (using a κ of $10^{-2} \text{ cm}^2 \text{ s}^{-1}$). The Makalu granite in the Himalayan range has been recently dated at about 21 Myr²⁴. This is evidence that melting conditions have been reached in the Tibetan crust after India collided with Asia.

From the value of 146 mW m^{-2} and the maximum range in corrections, it is possible to give a rough estimate of the depth to the anomalous body. Assuming steady-state conditions and a standard radioactivity model (see ref. 19), and taking the 600°C isotherm as a reference for crustal solidus temperatures

(for hydrated material such as sediments), we find that this depth must be between 10 and 25 km. Allowing for transient effects, these estimates must be considered as upper bounds. A conservative conclusion is therefore that the heat source must be shallower than 25 km. Such small crustal depths would indeed yield a sharp horizontal variation of heat flow in the vicinity of the body edge. The horizontal distribution of heat flow allows some further constraints on the timing and depth of the intrusive or melting event which will be developed in a future paper.

Our data yield no evidence for any significant heat flow variation over a distance of ~30 km (Fig. 6). This suggests that the heat source is of large horizontal dimensions. If this source is also the cause of the widespread geothermal activity of the plateau, it is not surprising that it has large horizontal dimensions. This would agree with geological observations made in old continental collision zones which typically exhibit tabular granitic formations extending over whole provinces²⁴.

Sharp horizontal heat flow variations are reminiscent of other tectonically active regions such as the southwestern United States^{25,26} or geothermal areas²⁷. Finally, note that the heat flow increase occurs at a distance of about 230 km from the main locale of underthrusting (Fig. 6). This is typical of subduction environments where high heat flows and volcanic activity show up abruptly at similar distances from trenches²⁸.

The measurements reported here suggest that regional melting conditions are reached at relatively shallow crustal depths in Tibet. This represents a powerful constraint for thermo-mechanical models of continental collisions.

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- Chen, W. P. & Molnar, P. *J. geophys. Res.* **86**, 5937-5962 (1981).
- Wang Chi-Yuen, Shi Yao-Lin & Wen Hu-Zhou *J. geophys. Res.* **87**, 2949-2957 (1982).
- Zeitler, P. K., Johnson, N. M., Naeser, C. W. & Tahirkheli, R. A. K. *Nature* **298**, 255-257 (1982).
- Wang Ji-Yang et al. *J. Volcan. geotherm. Res.* **9**, 57-76 (1981).
- Chapman, D. S. & Pollack, H. N. *Earth planet. Sci. Lett.* **28**, 23-32 (1975).
- Hart, S. R. & Steinhardt, J. S. *Science* **149**, 1499-1501 (1965).
- England, P. C. *Earth planet. Sci. Lett.* **39**, 427-434 (1978).
- Finckh, P. *Bull. geol. Soc. Am.* **92**, 452-514 (1981).
- Allis, R. G. & Garland, C. D. *Can. J. Earth Sci.* **16**, 1951-1964 (1979).
- Geological Map of the Tibetan Plateau*, 1/1,500,000 (Chengdu, China, 1981).
- Von Herzen, R. P. & Maxwell, A. E. *J. geophys. Res.* **64**, 1557-1563 (1959).
- Tapponnier, P., Mercier, J. L., Armijo, R., Tong Li-Han & Zhou-Ji *Nature* **294**, 410-414 (1981).
- Carlsaw, H. S. & Jaeger, J. C. *Conduction of Heat in Solids* (Oxford University Press, 1959).
- Guo, S. in *Symposium on Qinghai-Xizang (Tibet) Plateau*, Beijing, 13-14 (Gordon & Breach, New York, 1980).

- Allègre, C. J. et al. *Nature* **307**, 17-22 (1984).
- Blatt, H., Middleton, G. & Murray, R. *Origin of Sedimentary Rocks* (Prentice Hall, Englewood Cliffs, 1980).
- England, P. C. & Richardson, S. W. *Geophys. J. R. astr. Soc.* **62**, 421-437 (1980).
- Houseman, G., McKenzie, D. & Molnar, P. *J. geophys. Res.* **86**, 6115-6132 (1981).
- Scater, J. G., Jaupart, C. & Galson, D. *Rev. Geophys. Space Phys.* **18**, 269-311 (1980).
- Vidal, P., Cocherie, A. & Lefort, P. *Geochim. cosmochim. Acta* **46**, 2279-2292 (1982).
- Van Ngoc, P., Boyer, D. & Therme, P. *Terra Cognita* **3**, 270 (1983).
- Scharer, U. *Earth planet. Sci. Lett.* in the press (1983).
- Scharer, U., Xu, R.-H. & Allègre, C. J. *Earth planet. Sci. Lett.* in the press (1983).
- Nielson, D. L., Clark, R. G., Lyons, J. B., England, E. J. & Borns, D. J. *Geol. Soc. Am. Mem.* **146**, 301-318 (1976).
- Warren, A. E., Sclater, J. G., Vacquier, V. & Roy, R. F. *Geophysics* **34**, 463-478 (1969).
- Bodell, J. M. & Chapman, D. S. *J. geophys. Res.* **87**, 2869-2884 (1982).
- Blackwell, D. D. & Baag, C. G. *Geophysics* **38**, 941-956 (1973).
- Blackwell, D. D., Bowen, R. G., Hull, D. A., Riccio, J. & Steele, J. L. *J. geophys. Res.* **87**, 8735-8754 (1982).

Evolution of the genes for the β subunits of human chorionic gonadotropin and luteinizing hormone

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Nucleotide sequence comparisons of the single gene for the human luteinizing hormone gene β subunit with two of the seven genes for the human chorionic gonadotropin β subunit suggest that the β human chorionic gonadotropin genes have evolved from an ancestral β luteinizing hormone gene by a series of selected changes with very little neutral drift. Moreover, the 24 amino acid carboxy-terminal extension of the human chorionic gonadotropin β subunit appears to have arisen by a single base deletion that incorporated the 3'-untranslated region of the ancestral β luteinizing hormone gene into the coding region.

HUMAN chorionic gonadotropin (HCG) and luteinizing hormone (LH) are two members of the glycoprotein hormone family. The two hormones are very similar both functionally and structurally, although they are expressed in different tissues and at different developmental stages. Luteinizing hormone is made in the pituitary and has a central role in promoting spermatogenesis and ovulation by stimulating the testes and ovaries to synthesize steroids. In contrast, HCG is made by the first trimester placenta and stimulates the ovaries to synthesize the steroids that are essential for the maintenance of pregnancy. Nevertheless, both hormones bind to the same testicular and ovarian receptors (for a review of the biochemistry of these hormones, see ref. 1).

In common with the other members of the glycoprotein hormone family, HCG and LH are dimeric with two dissimilar, noncovalently associated subunits, α and β . The α subunit is common to all the glycoprotein hormones¹ and, in humans, is encoded by a single gene which has been isolated and characterized²⁻⁴. Unlike the α subunit, the β subunits are unique to each hormone and confer biological specificity. However, in keeping with their closely related biological functions, β -HCG and β -LH have an 82% amino acid sequence homology⁵⁻⁹.

We isolated a cDNA clone for the β subunit of HCG¹⁰ and used this to examine the structure and chromosomal organization of the genes for the β subunits of HCG and LH. We found that the β -HCG and β -LH genes were linked¹¹ and that the latter was encoded by a single gene¹². In contrast, we found that β -HCG is encoded by seven genes or pseudogenes^{11,12} which, together with the single β -LH gene, were shown to have an unusual structural organization of genes arranged in tandem and inverted pairs¹¹. Figure 1 summarizes these data.

We present here the complete nucleotide sequences for the single β -LH gene and for two of the β -HCG genes, those numbered 5 and 6, which were chosen because they form one of the two inverted gene pairs (see Fig. 1) and we were interested in the possible implications of this symmetrical arrangement for gene expression. The strategies used to determine the sequences are shown in Fig. 2 and the sequences, aligned with respect to each other, are shown in Fig. 3. Comparison of these sequences suggests that the β -HCG gene evolved from an ancestral β -LH gene by a series of selected changes with very little neutral genetic drift. The sequence comparison also shows that the carboxy-terminal extension of the β -HCG subunit protein arose, as we previously predicted¹⁰, by a readthrough event in which the 3'-untranslated region of the β -LH gene became

incorporated into the coding region. This readthrough could have been caused by a single base deletion.

Origin of the HCG gene

Luteinizing hormone is synthesized in the pituitaries of a wide range of species. In contrast, chorionic gonadotropin is found only in the placenta of certain mammals such as horses, baboons and humans^{1,13,14}, and is thus considered to be the most recently evolved member of the glycoprotein hormone gene family. Due to the strong structural and biological similarity between LH and HCG, it has been suggested previously that the β -HCG gene arose directly from a β -LH or β -LH-like ancestral gene¹⁵.

The β subunit of HCG is 24 amino acids longer than the β subunit of LH. Alignment of the two β subunit amino acid sequences shows that the extra 24 amino acids of the HCG β subunit form a carboxy-terminal extension that is unique to β -HCG. We suggested previously¹⁰ that this carboxy-terminal extension resulted from a readthrough event in which the β -HCG gene evolved from its β -LH gene ancestor by incorporation of the 3'-untranslated region into the coding sequence. This suggestion was based on the observation that the cDNA

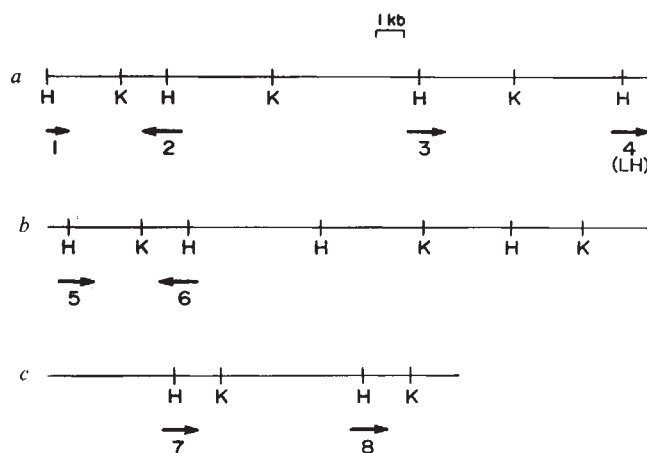


Fig. 1 Organization of the three groups, a, b and c, of cloned sequences that hybridize to the β human chorionic gonadotropin cDNA probe. The positions of the eight genes (4 is the single β luteinizing hormone gene) are shown by arrows pointing in the putative directions of transcription. Locations of sites for the restriction enzymes *Hind*III (H) and *Kpn*I (K) are shown. The identification of these genes was established in refs 11 and 12. The map is adapted from ref. 11.

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Table 1 Gene sequence comparisons

Gene pairs	Replacement changes		Silent changes		Intron changes	
	No.	%	No.	%	No.	%
LH:CG5	19/296.7	6±2	7/101.3	7±3	24/545	4±1
LH:CG6	19/296.7	6±2	8/101.3	8±3	32/545	6±1
CG5:CG6	1/369	0.3±0.3	1/123	1±1	18/545	3±1
HGH:HPL	33.5/482	7±1	18.5/161	11±3	72/726	10±1
RI:RII	8/249.2	3±1	15/80.8	19±5	21/99	21±5
RBG:MBG	35/330	11±2	49/110	44±6	120/267	45±4

Genes compared: LH, β luteinizing hormone; CG5, β human chorionic gonadotropin gene 5; CG6, β human chorionic gonadotropin gene 6; HGH, human growth hormone; HPL, human placental lactogen; RI, rat preproinsulin gene I; RII, rat preproinsulin gene II; RBG, rabbit β globin; MBG, mouse β globin. The values for the RI:RII and RBG:MBG comparisons are taken from ref. 20, all other values were calculated by the method given in ref. 20. The fractions (%) are the number of actual substitutions divided by the number of potential substitutions of a given type. The standard deviations were estimated as the square root of [the fractional change (%) divided by the number of potential changes]. The data are not corrected for possible multiple substitutions. The two LH:CG comparisons were made over the homologous coding regions from the start codons at position 553 up to, but not including, the single base deletion in the CG genes that is responsible for the frameshift. The CG5:CG6 comparison is over the 164 residue signal sequence and mature protein encoding regions. The HGH:HPL comparison is over their 210 residue signal sequence and mature protein encoding regions.

for the β subunit of HCG had a particularly short 3'-untranslated region of 16 nucleotides and that the termination codon of β -HCG was the TAA sequence of the polyadenylation signal AATAAA¹⁶.

We can now identify the nucleotide changes that caused the readthrough. A single base deletion at position 1,540 puts the β -HCG sequence out of frame with the last seven codons of β -LH and allows the 3'-untranslated region to become incorporated into the coding region. In addition to this, a two base pair (bp) insertion at positions 1,612 and 1,613 in the β -HCG sequence allows the carboxy-terminal extension to continue to the termination codon, TAA. Without this 2 bp insertion, the β -HCG carboxy-terminal extension would be eight amino acids shorter, ending with a TGA termination codon. If the 2-base insertion occurred first, it would have been a neutral mutation as the inserted bases would still be part of the 3'-untranslated region. If the single base deletion occurred first, then the β -HCG coding sequence has undergone two carboxy-terminal extensions, but we consider this unlikely. Nevertheless, by either mechanism, the primary event that led to the incorporation of the 3'-untranslated region into the coding region was the single base pair deletion. We note that the β -LH termination codon, put out of frame in the β -HCG gene, has been lost by a single base change, but we do not think that that could have been the initial event leading to the carboxy-terminal extension because there is no other terminator in the 3'-untranslated region in frame with it.

Thus, the β subunit of HCG has acquired 8 amino acids by translation in a new reading frame, and 24 amino acids from a previously untranslated region. This has occurred without any

substantial change other than the frameshift mutations as β -HCG genes 5 and 6 are 90% homologous with the β -LH gene over the region encoding the carboxy-terminal extension, nucleotides 1,541–1,634. The function of this carboxy-terminal extension is not known as HCG appears to bind to the same membrane receptors and to elicit the same response as LH. However, HCG is 10 times more stable than LH, and it has been suggested that the carboxy-terminal extension has a role in this (see ref. 13 for a review).

Nucleotide changes

We suggested previously^{11,12} that the β -HCG gene arose by a duplication of an ancestral gene. This duplication was followed by a series of changes, including the frameshift mutations, which generated the new β -HCG function. Once this new function was established, the β -HCG gene itself duplicated and rearranged to give the present complex organization (see Fig. 1). The nucleotide sequences we have established here provide information on the series of changes that took place as the newly duplicated ancestral gene evolved into that for β -HCG.

To compare the nucleotide sequence differences between the β -LH gene and the two β -HCG genes 5 and 6, we have calculated the per cent divergence between the introns, and between replacement and silent substitution sites across the homologous coding regions. These data are shown in Table 1. Table 1 also shows similar calculations for a selection of other gene pairs, including the two β -HCG genes themselves, human growth hormone (HGH) and human placental lactogen (HPL) genes^{17–19}, the two rat preproinsulin genes I and II²⁰, and the rabbit and mouse β -globin genes^{21,22}.

Fig. 2 Nucleotide sequencing strategy for the β -HCG genes 5 and 6 (CG5 and CG6) and the β -LH gene. Arrows mark the restriction sites sequenced from and the extent of the individual sequence determinations, the dotted portions of the arrows indicating where the sequence was not read immediately adjacent to a labelled site. Restriction enzyme sites used in the sequence determination are marked. A, *Ava*I; Ap, *Apa*I; B, *Bgl*I; D, *Dde*I; F, *Hinf*I; H, *Hinc*II; H3, *Hind*III; N, *Nco*I; Na, *Nae*I; P, *Pvu*II; (R), *Eco*RI (generated by an *Eco*RI linker); Rs, *Rsa*I; S, *Stu*I; Sa, *Sac*I. The nucleotide sequence of β -LH gene shown in Fig. 3 includes the area marked by solid arrows. The nucleotide sequences of β -HCG genes 5 and 6 shown in Fig. 3 begin about 100 bp after the solid arrows above. Both the chain termination method (a) and the chemical degradation method (b) were used. For chain termination sequencing³⁶, purified fragments were blunt-ended and cloned into the unique *Sma*I site of M13mp8³⁷. A 17 bp synthetic primer was purchased from P-L Biochemicals. Chemical sequencing³⁸ was performed on fragments labelled at their 3' ends with [α -³²P]-deoxynucleoside triphosphates and the Klenow fragment of DNA polymerase I. All restriction enzyme sites were overlapped in the β -HCG gene 5, but in the cases of β -HCG gene 6 and the β -LH gene, the overlap at one and two sites respectively was done by analogy with the β -HCG gene 5.

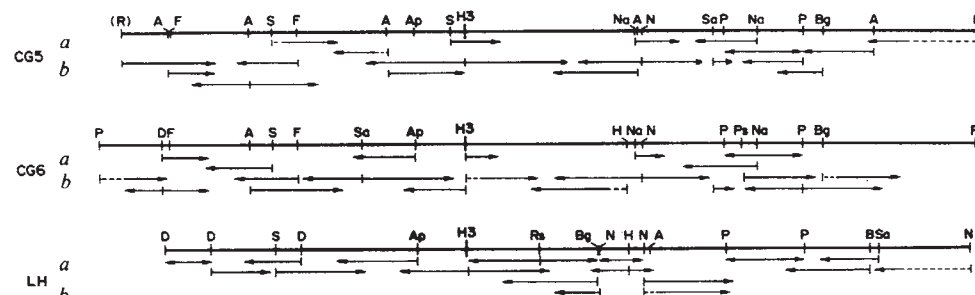


Fig. 3 The complete nucleotide sequences of the β -HCG genes 5 and 6 (CG5 and CG6) and the β -LH gene. The three sequences have been aligned with gaps where there are deletions and insertions, and the nucleotide sequence numbers, immediately above the nucleotide sequences, are not adjusted for deletions. In the alignment of the nucleotide sequences, bold face letters indicate positions where one of the three sequences differs from the other two. The two asterisks at nucleotides 190 and 191 identify the proposed transcriptional start for β -HCG (unpublished data), and the single asterisk at nucleotide 1,654 marks the polyadenylation site. The amino acid sequence of β -LH is shown above the nucleotide sequence. For the β -HCG genes 5 and 6, only the predicted amino acids that differ from those of β -LH are shown below the nucleotide sequences. Amino acid numbers are placed immediately above amino acid names, and negative numbers refer to amino acids in the signal peptide. The β -HCG genes 5 and 6 introns were located by comparison with the β -HCG cDNA sequence¹⁰. The β -LH gene introns were located by homology with the β -HCG gene sequences. The sequences at the intron-exon boundaries show the expected homologies with those in other systems³⁹⁻⁴¹. The β -LH amino acid sequence derived from our nucleotide sequence differs from the published sequence^{5,9}, in that there is a Met-Met and a Val-Val at positions 41-42 and 55-56, respectively, rather than the single methionine and valine residues previously reported^{5,9}, bringing the β -LH protein sequence into register with that of β -HCG. Also, the carboxy-terminus is seven amino acids longer than previously reported⁹, making the human β -LH sequence more homologous to the ovine, porcine and bovine sequences⁴²⁻⁴⁴ and

LH	AAGGGAGAGGTGGGGCTCGGGCTTAATCCCTCCTTGGGGGCATCGGGTCAAGTGGCTTCCTGGCAGCAGTACAGGGGAGACCTCTCTCACTGGG	50	100
CG5	AAGGGAGAGGTGGGGCTCGGGCTGAATCCCTCCTTGGGGGCATCGGGTCAAGTGGCTTCCTGGCAGCAGTACAGGGGAGGCCCTCTCTCACTGGG		
CG6	AAGGGAGAGGTGGGGCTCGGGCTGAATCCCTCCTTGGGGGCATCGGGTCAAGTGGCTTCCTGGCAGCAGTACAGGGGAGACCTCTCTCACTGGG		
LH	CAGAAGCTAAGTCCGAAGCAGCGCCCTCCTGTTAGGTGGAGTGTGGTGCAGGAAGCCTCAAGT	150	200
CG5	CAGAAGCTAAGTCCGAAGCAGCGCCCTCCTGTTAGGTGGAGTGTGGTGCAGGAAGCCTCAAGT		
CG6	CAGAAGCTAAGTCCGAAGCAGCGCCCTCCTGTTAGGTGGAGTGTGGTGCAGGAAGCCTCAAGT		
LH	TGGGGTCAATGGCTCCTCTGGCTCCCAAGACCCCAACATGGCAGAGGAGGCCCTTCTACACCTACTCCTCTGTGCTTCCAGCCTCGACTAGTCCCTA	250	300
CG5	TGGGGTCAATGGCTCCTCTGGCTCCCAAGACCCCAACATGGCAGAGGAGGCCCTTCTACACCTACTCCTCTGTGCTTCCAGCCTCGACTAGTCCCTA		
CG6	TGGGGTCAATGGCTCCTCTGGCTCCCAAGACCCCAACATGGCAGAGGAGGCCCTTCTACACCTACTCCTCTGTGCTTCCAGCCTCGACTAGTCCCTA		
LH	GCACCTGACAACTGAGTCTCTGAGGTCACTTACCGTGGTCTCTGCTCTACCTCTGGCGTAGACCCGTAGGGGAGAGGGCTGGGGCAGCTCTGCTGAGC	350	400
CG5	GCACCTGACAACTGAGTCTCTGAGGTCACTTACCGTGGTCTCTGCTCTACCTCTGGCGTAGACCCGTAGGGGAGAGGGCTGGGGCAGCTCTGCTGAGC		
CG6	GCACCTGACAACTGAGTCTCTGAGGTCACTTACCGTGGTCTCTGCTCTACCTCTGGCGTAGACCCGTAGGGGAGAGGGCTGGGGCAGCTCTGCTGAGC		
LH	CACCTCTGCGCTCCTCTGGCTTGTGACCTCTCGCCCCGGGGATTAGTGTCCAGGTACCCCAAGGCATCTTATCACCTCTGGTGGCCCTTGGCGCCC	450	500
CG5	CACCTCTGCGCTCCTCTGGCTTGTGACCTCTCGCCCCGGGGATTAGTGTCCAGGTACCCCAAGGCATCTTATCACCTCTGGTGGCCCTTGGCGCCC		
CG6	CACCTCTGCGCTCCTCTGGCTTGTGACCTCTCGCCCCGGGGATTAGTGTCCAGGTACCCCAAGGCATCTTATCACCTCTGGTGGCCCTTGGCGCCC		
LH	CCACAACCCCGAGGTATAAAGCCAGATACACGAGGAGGGGATGCACCAAGGATGGAGATGCTCCAGGTAAAGCTGCAGGGGCCCTGGGCACCTTCCACC	550	600
CG5	CCACAACCCCGAGGTATAAAGCCAGGTACACGAGGAGGGGATGCACCAAGGATGGAGATGCTCCAGGTAAAGCTGCAGGGGCCCTGGGCACCTTCCACC		
CG6	CCACAACCCCGAGGTATAAAGCCAGGTACACGAGGAGGGGATGCACCAAGGATGGAGATGCTCCAGGTAAAGCTGCAGGGGCCCTGGGCACCTTCCACC		
LH	TCCTTCCAGGCCATCACTGGCATGAGAAGGGGAGACCCGTGTGAGTGTGGAAGGAGGCCCTCTTCTGGAGGGGCGTGACCCCAAGTAAAGCTTCAAGTG	650	700
CG5	TCCTTCCAGGCCATCACTGGCATGAGAAGGGGAGACCCGTGTGAGTGTGGAAGGAGGCCCTCTTCTGGAGGGGCGTGACCCCAAGTAAAGCTTCAAGTG		
CG6	TCCTTCCAGGCCATCACTGGCATGAGAAGGGGAGACCCGTGTGAGTGTGGAAGGAGGCCCTCTTCTGGAGGGGCGTGACCCCAAGTAAAGCTTCAAGTG		
LH	GGGCAGTTCCTGAGGGTGGGGATCTGAAATGTTGGGGATCTCAGGTCTCTGGGCTGTGGGGTGGGCTCTGAAAGGCAGGTGTCCGGGTGGTGGGTCT	750	800
CG5	GGGCAGTTCCTGAGGGTGGGGATCTGAAATGTTGGGGATCTCAGGTCTCTGGGCTGTGGGGTGGGCTCTGAAAGGCAGGTGTCCGGGTGGTGGGTCT		
CG6	GGGCAGTTCCTGAGGGTGGGGATCTGAAATGTTGGGGATCTCAGGTCTCTGGGCTGTGGGGTGGGCTCTGAAAGGCAGGTGTCCGGGTGGTGGGTCT		
LH	GAATAGGAGATGCCGGAAGGGTCTCTGGGCTTTTGTGGGTGGTGTACACCGGGATGGGAAGGCCAGGACTCGGGGCTCGGGTCTCAGACCCGGGTGA	850	900
CG5	GAATAGGAGATGCCGGAAGGGTCTCTGGGCTTTTGTGGGTGGTGTACACCGGGATGGGAAGGCCAGGACTCGGGGCTCGGGTCTCAGACCCGGGTGA		
CG6	GAATAGGAGATGCCGGAAGGGTCTCTGGGCTTTTGTGGGTGGTGTACACCGGGATGGGAAGGCCAGGACTCGGGGCTCGGGTCTCAGACCCGGGTGA		
LH	AGCAGTGTCTTGTCTCCAGGGGCTGCTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCT	-15	-1
CG5	AGCAGTGTCTTGTCTCCAGGGGCTGCTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCT		
CG6	AGCAGTGTCTTGTCTCCAGGGGCTGCTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCT		
LH	AsnAlaIleLeuAlaValGluLysGluGlyCysProValCysIleThrValAsnThrThrIleCysAlaGlyTyrCysProThrMet	20	41
CG5	AATGCCATCTCTGGCTGTGGAGAAGGAGGGCTGCCCGGTGTGCATCACCCTCAACACCCACCATCTGTGGCGGCTACTGCCACCATGGTGGAGCTGCCCGG		
CG6	AATGCCATCTCTGGCTGTGGAGAAGGAGGGCTGCCCGGTGTGCATCACCCTCAACACCCACCATCTGTGGCGGCTACTGCCACCATGGTGGAGCTGCCCGG		
LH	GGCCGGGGCAGATGCTGCCACCTCAGGGGCCAGACCCACAGAGGAGGGGAGGAGGTGGTCTGCCTCTCTGGCTTGGGCTGGGGATGGGGTGTG	1150	1200
CG5	GGCCGGGGCAGATGCTGCCACCTCAGGGGCCAGACCCACAGAGGAGGGGAGGAGGTGGTCTGCCTCTCTGGCTTGGGCTGGGGATGGGGTGTG		
CG6	GGCCGGGGCAGATGCTGCCACCTCAGGGGCCAGACCCACAGAGGAGGGGAGGAGGTGGTCTGCCTCTCTGGCTTGGGCTGGGGATGGGGTGTG		
LH	GGAGGGCAGGAACAGAGGGCTTCTGGGCTCTGAGTCTGAGACCTGTGGGGTCACTTGGGAGCTCAGCTGAGGCGCTGGCTTAGGCACATGCTCATTC	1250	1300
CG5	GGAGGGCAGGAACAGAGGGCTTCTGGGCTCTGAGTCTGAGACCTGTGGGGTCACTTGGGAGCTCAGCTGAGGCGCTGGCTTAGGCACATGCTCATTC		
CG6	GGAGGGCAGGAACAGAGGGCTTCTGGGCTCTGAGTCTGAGACCTGTGGGGTCACTTGGGAGCTCAGCTGAGGCGCTGGCTTAGGCACATGCTCATTC		
LH	CCCCACTCACACGGCTTCCAGACCCCGGTGCTGACAGGCTTCCGCGCCCTGCTCAGGTGGTGTGCACTACCGTATGTCAGTGTGCGCTTCCAGTCCATCC	42	60
CG5	CCCCACTCACACGGCTTCCAGACCCCGGTGCTGACAGGCTTCCGCGCCCTGCTCAGGTGGTGTGCACTACCGTATGTCAGTGTGCGCTTCCAGTCCATCC		
CG6	CCCCACTCACACGGCTTCCAGACCCCGGTGCTGACAGGCTTCCGCGCCCTGCTCAGGTGGTGTGCACTACCGTATGTCAGTGTGCGCTTCCAGTCCATCC		
LH	rgLeuProGlyCysProArgGlyValAspProValValSerPheProValAlaLeuSerCysArgCysGlyProCysArgArgSerThrSerAspCysG1	80	100
CG5	GGCTCCCTGGCTGCCCGCGTGGCGTGGACCCCGTGGTCTCTTCCCTGTGGCTCTCAGTGTGCTGTGGACCTTCCGCGCGCAGCACCCTTGACTGTGG		
CG6	GGCTCCCTGGCTGCCCGCGTGGCGTGGACCCCGTGGTCTCTTCCCTGTGGCTCTCAGTGTGCTGTGGACCTTCCGCGCGCAGCACCCTTGACTGTGG		
LH	GGGTCCTCCAGGACCCCTTACCTTGTGATGACCCCGC	121	
CG5	GGGTCCTCCAGGACCCCTTACCTTGTGATGACCCCGC		
CG6	GGGTCCTCCAGGACCCCTTACCTTGTGATGACCCCGC		
LH	CCTGGAGCCCT GACACCCCGATCTCCCAATAAAGGCTTCTCAATCCGCACTCTGGCAGTATC	1650	
CG5	CCCGGGGCCCTCGGACACCCCGATCTCCCAATAAAGGCTTCTCAATCCGCACTCTGGCAGTATC		
CG6	CCCGGGGCCCTCGGACACCCCGATCTCCCAATAAAGGCTTCTCAATCCGCACTCTGGCAGTATC		

121 residues, rather than 112, in length. The amino acid sequences predicted by the β -HCG genes 5 and 6 sequences are identical with the exception of position 117, which is aspartic acid in gene 5 and alanine in gene 6. This position is aspartic acid in both the published amino acid sequences from two groups^{7,8} and in our cDNA sequence¹⁰. The β -HCG genes 5 and 6 have a single silent change in codon 4; our cDNA sequence¹⁰ and β -HCG gene 5 agree. The nucleotide sequence of the β -HCG gene 5 is identical with that of the published cDNA sequence with two exceptions: nucleotide position 529 is a C in the gene sequence and a G in the cDNA sequence (this nucleotide is close to the 5' end of the cDNA clone, and presumably was misincorporated by reverse transcriptase, as has been observed in other systems⁴⁵), and nucleotide position 1,589 is a T in the gene sequence and a C in the cDNA sequence (this is a silent third position change and is an error in the published cDNA sequence¹⁰).

Striking differences emerge from these comparisons. For the preproinsulin and β -globin gene pairs, the rates of intron and silent position divergence are similar within each pair, and are four to seven times greater than the rate of replacement changes. Similar results have been obtained with a further 42 gene pairs encoding identical functions and a further 4 gene pairs encoding different functions^{23,24}. In marked contrast, the replacement and silent sites of the two β -HCG genes have diverged from those of the β -LH gene at similar rates, between 6 and 8%, whilst the introns of each pair have diverged slightly less, between 4 and 6%. Thus, unlike most other gene pairs, the replacement, silent and intron changes have all occurred at approximately equal rates. The introns and silent sites of the HGH/HPL gene pair have also diverged at rates that are only slightly higher than the rate of replacement changes. It is interesting that the HGH/HPL gene pair is analogous in this respect to the β -HCG/ β -LH gene pair: both proteins in each of the pairs have similar functions, but one (HGH/HPL) is synthesized in the pituitary while the other (β -HCG/ β -LH) is synthesized in the placenta. A high rate of replacement, compared with neutral, changes has also been observed in a pair of rabbit globin alleles^{21,25,26} and a pair of immunoglobulin light-chain genes²⁷, and in cDNA fragments encoding the carboxy-terminal thirds of two mouse H-2^d antigens²⁸.

Discussion

How can we explain the high rate of replacement, compared with neutral, changes between the β -LH/ β -HCG and the HGH/HPL gene pairs? One theory of evolution holds that observed changes in protein structure are spread through the population by selection²⁹⁻³¹. In support of this hypothesis, Perler *et al.*²³ cite the two rabbit β -globin alleles^{21,25,26}, between which there are four replacement changes with no silent changes and only two intron changes, as a clear example of selection. They assumed that these changes were fixed so rapidly by selection that there was no opportunity for neutral changes to appear. This could be the case for the reported immunoglobulin light-chain²⁷ and H-2^d antigen²⁸ gene pair comparisons, although the generation of polymorphisms in these systems may be a specialized and, at least in the case of the immunoglobulin light chains, perhaps distinct mechanism. The selection argument of Perler *et al.*²³ can also be applied to the evolution of the new functions in the β -HCG/ β -LH and HGH/HPL gene pairs.

An alternative interpretation of the high rate of replacement,

compared with neutral, changes in the evolution of the β -HCG from a β -LH gene is that genes evolve first by fixation of the new function (selection), followed by accumulation of neutral changes (drift). By this hypothesis, the evolution of β -HCG is the most recent event yet analysed, more recent than the evolution of HPL. Like the β -chorionic gonadotropin and β -LH genes, the GH and PL genes diverged late in mammalian radiation³², but which gene family diverged first is not known because systematic cross-species protein comparisons either for chorionic gonadotropin or PL have not been done yet.

The neutral mutation-random drift theory³³⁻³⁵ holds that most observed changes in amino acid sequence are neutral and are fixed in the population by random drift. Unless the β -HCG genes duplicated very recently, it is difficult to imagine how so many replacement changes could have taken place between the β -HCG and β -LH genes by neutral drift, given that so few replacement changes have been allowed between the two β -HCG genes. It is unlikely that the β -HCG genes duplicated recently compared with the original β -LH gene duplication, because the rate of divergence of the intron sequences of β -HCG genes 5 and 6 approaches those of the β -LH/ β -HCG gene pairs.

From the rapid rate of replacement relative to neutral changes in the evolution of β -HCG from β -LH, and the low level of replacement relative to intron changes between the two β -HCG genes, we conclude that selection of a new function is the mechanism most consistent with the observed sequence differences. If stability is truly the only difference between HCG and LH, then the changes across the homologous coding region, and not just the carboxy-terminal extension, must be involved. However, we believe that there may be other, as yet unidentified, functional differences between chorionic gonadotropin and LH that require the observed coding differences. Moreover, because the divergence between the two β -HCG intron sequences approaches the divergence between the introns of the two β -LH/ β -HCG gene pairs, we suggest that the β -HCG gene duplications and rearrangements occurred soon after the establishment of the new β -HCG function.

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- Pierce, J. G. & Parsons, T. F. A. *Rev. Biochem.* **50**, 465-495 (1981).
- Fiddes, J. C. & Goodman, H. M. *Nature* **281**, 351-356 (1979).
- Fiddes, J. C. & Goodman, H. M. *J. molec. appl. Genet.* **1**, 3-18 (1981).
- Boothby, M., Ruddon, R. W., Anderson, C., McWilliams, D. & Boime, I. *J. biol. Chem.* **265**, 5121-5127 (1981).
- Shome, B. & Parlow, A. F. *J. clin. Endocr. Metab.* **36**, 618-621 (1973).
- Morgan, F. J., Birken, S. & Canfield, R. E. *J. biol. Chem.* **250**, 5247-5258 (1975).
- Keutmann, H. T. & Williams, R. M. *J. biol. Chem.* **252**, 5393-5397 (1977).
- Birken, S. & Canfield, R. E. *J. biol. Chem.* **252**, 5386-5392 (1977).
- Keutmann, H. T., Williams, R. M., Ryan, R. J. *Biochem. Biophys. Res. Commun.* **90**, 842-848 (1979).
- Fiddes, J. C. & Goodman, H. M. *Nature* **286**, 684-687 (1980).
- Boorstein, W. R., Vamvakopoulos, N. C. & Fiddes, J. C. *Nature* **300**, 419-422 (1982).
- Talmadge, K., Boorstein, W. R. & Fiddes, J. C. *DNA* **2**, 279-287 (1983).
- Canfield, R. E., Birken, S., Morse, J. H. & Morgan, F. J. in *Peptide Hormones* (ed. Parsons, J. A.) 299-315 (University Park, Baltimore, 1978).
- Ward, D. N., Moore, W. T. Jr & Burleigh, B. D. *J. Protein Chem.* **1**, 263-280 (1982).
- Dayhoff, M. O. in *Atlas of Protein Sequences and Structure* Vol. 5, Suppl. 2, 122 (National Biomedical Research Foundation, Washington 1976).
- Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211-214 (1976).
- Goodman, H. M. *et al.* in *Mobilization and Reassembly of Genetic Information* (eds Scott, W. A., Werner, R., Joseph, D. R. & Schulz, J.) 155-179 (Academic, New York, 1980).
- DeNoto, F. M., Moore, D. D. & Goodman, H. M. *Nucleic Acids Res.* **9**, 3719-3730 (1981).
- Seeburg, P. H. *DNA* **1**, 239-249 (1982).
- Lomedico, P. *et al. Cell* **18**, 545-558 (1979).
- Elstradiatis, A., Kafatos, F. C. & Maniatis, T. *Cell* **10**, 571-585 (1977).
- van den Berg *et al. Nature* **276**, 37-44 (1978).
- Perler, F. *et al. Cell* **20**, 555-566 (1980).
- Miyata, T., Yasunga, T. & Nishida, T. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7328-7332 (1980).
- Hardison, R. C. *et al. Cell* **18**, 1285-1297 (1979).
- van Ooyen, A., van den Berg, J., Mantei, N. & Weissmann, C. *Science* **206**, 337-344 (1979).
- Bernard, O., Hozumi, N. & Tonegawa, S. *Cell* **15**, 1133-1144 (1978).
- Bregere, F. *et al. Nature* **292**, 78-81 (1981).
- Clarke, B. *Science* **168**, 1009 (1970).
- Richmond, R. C. *Nature* **225**, 1025 (1975).
- Blundell, T. L. & Wood, S. P. *Nature* **257**, 197 (1975).
- Niall, H. D. in *Prolactin and Carcinogenesis* (ed. Griffiths, K.) 13-20 (Alpha Omega Alpha, Cardiff, 1972).
- Kimura, M. *Nature* **217**, 624-626 (1968).
- Kimura, M. & Ohta, T. *Nature* **229**, 467-469 (1971).
- Kimura, M. & Ohta, T. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2848-2852 (1974).
- Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
- Messing, J. & Vieira, J. *Gene* **19**, 269-276 (1982).
- Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Breathnach, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4853-4857 (1978).
- Catterall, J. F. *et al. Nature* **275**, 510-513 (1978).
- Breathnach, R. & Chambon, P. A. *Rev. Biochem.* **50**, 349-383 (1981).
- Liu, W.-K. *et al. J. biol. Chem.* **247**, 4365-4381 (1972).
- Sairam, M. R., Samy, T. S. A., Papkoff, H. & Li, C. H. *Archs Biochem. Biophys.* **153**, 572-586 (1972).
- Maghuin-Rogister, G. & Hennen, G. *Eur. J. Biochem.* **39**, 235-253 (1973).
- Richards, R. I. *et al. Nucleic Acids Res.* **7**, 1134-1146 (1979).

Recurrent bursts in GBS0526-66, the source of the 5 March 1979 γ -ray burst

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The results of the observations of GBS0526-66 during 1979¹⁻³ have been reviewed⁴ recently primarily in the context of its possible identification with the N49 supernova remnant in the Large Magellanic Cloud (LMC). Observations in 1981 and 1982, however, reveal a still more complex pattern of behaviour and show both that the nature of this source of the 5 March 1979 event and its true position in space are far from being clear. Observations from Veneras 13 and 14 reveal that GBS0526-66 continues to generate weak recurrent γ -ray bursts. Thus the transient object which during the 5 March burst behaved for several minutes as a flaring X-ray pulsar¹ has since then been exhibiting a peculiar similarity with a hard X-ray burster.

γ -Ray burst observations from Veneras 13 and 14 started in November 1981. The Konus instrument used is a modified version of the one flown on Veneras 11 and 12 (ref. 5). The time resolution and recording time essential for the burst time profile measurements have been improved. Apart from this, Venera 13 can now measure 16 energy spectra consecutively. While Venera 14 obtains eight consecutive spectra as before, the number of the energy channels in each spectrum has been increased to 30. In both cases the accumulation time for the first two spectra is 0.5 s; the subsequent spectra are averaged over 4 s, as before.

The first two recurrent bursts originating in GBS0526-66 were recorded on 1 December 1981 and 2 January 1982⁶. The time profiles and energy spectra of these events are shown in Fig. 1, the profiles being drawn with a 1/32 s time resolution. They reveal short rise and decay times. From the available recordings with a 1/256 s resolution one may conclude that the rise times here are $\tau_r < 30$ ms.

No spectral variability has been found within experimental accuracy. Therefore for GB811201 we present a net spectrum summed over three consecutive measurements 0.5, 0.5 and 4 s long, while for the shorter burst GB820102a the sum of the first two spectra accumulated in 0.5 s each is shown. The incident photon spectra were obtained by the usual procedure of unfolding the experimental energy loss spectra using the sensitivity matrix of our detectors. Figure 1 shows the spectra in energy units. The spectra are seen to approximate the relationship $dI(E)/dE \propto \exp(-E/kT)$ with the temperature kT , accordingly, 31 ± 2 and 26 ± 3 keV.

Ten more weak recurrent bursts from the same source have subsequently been recorded on the two spacecraft. The time profiles of some of these bursts are shown in Fig. 2. Because the spectra of these weak events were measured with a poor statistical accuracy, we do not present here the corresponding graphs. However, the data obtained clearly indicate that these spectra resemble one another and correspond to an emission temperature ≈ 35 keV.

Localization of these bursts was carried out by the usual procedure in the Konus experiment when recording an event at the two spacecraft⁵. The difference in the arrival times yielded a triangulation ring, the section containing the source being determined by analysing the anisotropic angular sensitivity of the detectors. Three events were detected only on Venera 13. The weak burst GB820921 did not trigger the recording on Venera 14. During the two other events, GB830321 and GB830405, the γ -ray burst detector on Venera 14 was switched

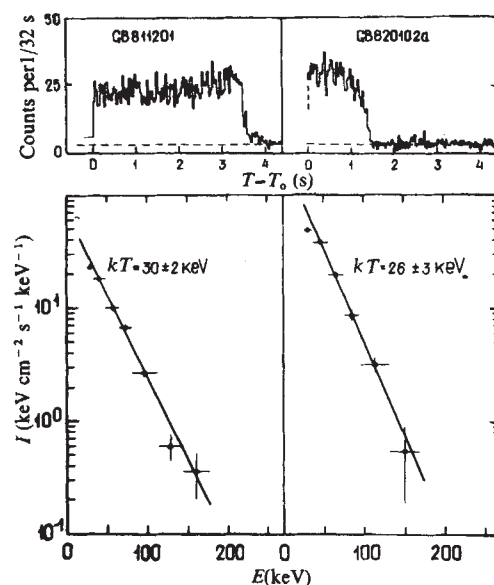


Fig. 1 Time profiles (top) and energy spectra (below) of the γ -ray bursts on 1 December 1981 and 2 January 1982. The horizontal dashed line in the time profiles shows the background level.

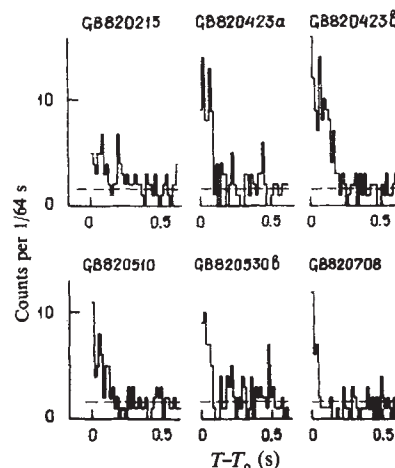


Fig. 2 Time profiles of the weak recurrent bursts from the GBS0526-66, February to July 1982.

off. These weak events were localized by the angular anisotropy technique with a low accuracy of $\sim 15^\circ$. The crude localization data, the shape of the time profiles and of the spectra suggest that these three events similarly belong to a general sequence of recurrent bursts.

The localization regions of 13 recurrent bursts from the GBS0526-66 observed in the Konus experiment on Veneras 11 and 12 in 1979^{7,8} and Veneras 13 and 14 in 1981-83 are presented in Fig. 3. All the localization regions are seen to intersect within a very small area of the sky which contains the most precise position for the 5 March 1979 event⁹ denoted with a cross.

The sequence of bursts of 1979 followed the powerful event of 5 March. Between our observations on Venera 11 and 12 and on Venera 13 and 14 there was a break from February 1980 to November 1981. The question may arise of whether the recurrent burst sequence of 1981-83 was preceded by as powerful an event. Apparently this was not the case. The 5

March event was detected on at least nine spacecraft⁴. An analogue of such an event could hardly have escaped observation. More probably, during the break in our observations several weak recurrent bursts occurred. However, a possible loss of a series of recurrent bursts cannot substantially alter the observed pattern of behaviour of the GBS0526-66. We see that the transient object which manifested itself in the 5 March event as a flaring X-ray pulsar reveals afterwards a certain similarity to a hard X-ray burster.

The major characteristics of the recurrent bursts are listed in Table 1 which contains the date and detection time T_0 of an event on the two spacecraft, burst duration T_b , emission temperature kT , peak count rate $N_{\max}$ and total intensity S . Also given are the coordinates α , δ of the triangulation ring centre, that is the direction of the line connecting the two spacecraft, the radius of the ring, its width $\theta \pm \Delta\theta$ and the minimum distance Δ to which the ring approaches the most precise position for the 5 March event.

These data reveal two remarkable features. First, the energy spectra of all the recurrent bursts have practically the same shape and are characterized by very close values of the temperature kT . Furthermore, they are very close to the spectrum of the soft component in the two-component spectrum of the narrow initial pulse in the 5 March 1979 burst and to that of the 8-s period pulsating radiation in its late stage⁸.

Second, the maximum count rate $N_{\max}$ in the recurrent bursts and, hence, the maximum luminosity of the source $L_{\max}$ also varies only slightly from event to event. Indeed, $N_{\max}$ does not differ within the error limits from the mean over all recurrent bursts which is 13 photons in 1/64 s. This count rate corresponds to the flux $I(>30 \text{ keV}) = 10^{-6} \text{ erg cm}^{-2} \text{ s}^{-1}$. Only in the first recurrent burst GB790306 does the maximum flux exceed this value by a factor of two. At the same time the scatter in the values of the total intensity S is much larger, namely by about

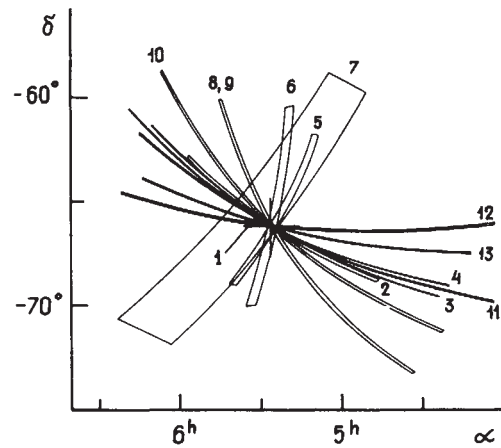


Fig. 3 Localization error boxes for the following events: 1, 5 March; 2, 6 March; 3, 4 April; 4, 24 April 1979; 5, 1 December 1981; 6, 2 January; 7, 15 February; 8 and 9, 23 April a and b; 10, 10 May; 11, 30 May; 12, 8 July; 13, 31 December 1982. The cross shows the most precise localization of the GBS0526-66 (ref. 9).

a factor of 40. These differences are determined essentially only by the different duration of the bursts T_b .

The 5 March 1979 event and views on its nature have been widely discussed (see review in ref. 4). New observations of the activity of this source show that the burst recurrence represents one of the most essential characteristics of its behaviour. Therefore these new data should prove to be important in the search for an explanation of the nature of the source. In particular, they provide a new argument against its identification with the N49 supernova remnant in the LMC at a distance $d = 55 \text{ kpc}$.

Table 1 Recurrent bursts from the 5 March 1979 event source

Event	Spacecraft	t_0 (UT) (h min s)	T_b (s)	kT (keV)	$N_{\max}$ (1/64s) ⁻¹	$S \times 10^{-6}$ (erg cm ⁻²)	Triangulation ring (deg)		
							α δ	θ	Δ
GB790305*	V-11	15 51 39.145	0.25	35 ± 5;	6×10^3	450	44.428	68.522 ± 0.030	0.026 ± 0.030
	V-12	15 51 44.352		520 ± 100			-2.747		
GB790306	V-11	6 17 25.201	1.5	31 ± 5	25	6.5	44.341	68.679 ± 0.085	0.009 ± 0.085
	V-12	6 17 30.457					-2.591		
GB790404	V-11	0 43 31.317	0.2	30 ± 10	15	0.7	43.708	77.142 ± 0.020	-0.006 ± 0.020
	V-12	0 43 37.938					6.015		
GB790424	V-11	18 27 18.241	0.2	30 ± 15	12	0.6	48.038	81.917 ± 0.012	0.013 ± 0.012
	V-12	18 27 24.730					11.971		
GB811201	V-13	9 18 02.424	3.5	30 ± 2	12	6.6	133.165	51.82 ± 0.14	0.08 ± 0.14
	V-14	9 17 59.132					-25.430		
GB820102a	V-13	17 11 11.109	1.5	26 ± 3	15	3.6	153.420	67.21 ± 0.17	0.04 ± 0.17
	V-14	17 11 09.707					-17.049		
GB820215	V-13	10 36 31.071	0.1	40 ± 15	7	0.15	163.819	130.6 ± 1.0	-0.7 ± 1.0
	V-14	10 36 33.279					49.482		
GB820423a	V-13	3 43 16.907	0.1	36 ± 10	13	0.2	205.788	122.489 ± 0.068	0.033 ± 0.068
	V-14	3 43 24.946					20.694		
GB820423b	V-13	16 29 21.315	0.15	45 ± 15	15	0.3	206.113	122.181 ± 0.068	0.010 ± 0.068
	V-14	16 29 29.350					20.275		
GB820510	V-13	13 27 55.921	0.1		10	0.15	215.867	113.998 ± 0.052	0.042 ± 0.052
	V-14	13 28 03.263					7.886		
GB820530b	V-13	12 46 13.968	0.1	35 ± 15	10	0.15	226.788	104.895 ± 0.043	0.017 ± 0.043
	V-14	12 46 19.305					-4.709		
GB820708	V-13	10 18 18.979	0.1	30 ± 15	12	0.15	251.669	87.438 ± 0.036	0.013 ± 0.036
	V-14	10 18 17.921					-26.155		
GB820921	V-13	18 37 54.138	0.1	27 ± 15	10	0.15			
GB821231	V-13	4 17 21.363	0.18	27 ± 10	14	0.3	61.798	85.235 ± 0.042	0.008 ± 0.042
	V-14	4 17 19.620					17.817		
GB830321	V-13	22 32 11.400	0.15	24 ± 10	12	0.2			
GB830405	V-13	7 58 07.667	1.1	31 ± 6	15	1.6			

* The data presented are for the initial burst pulse, whose spectrum consist of two (one hard and one soft) components. For the pulsating phase $S = 10^{-3} \text{ erg cm}^{-2}$ (ref. 7), $kT = 35.5 \pm 1.0 \text{ keV}$ (main pulse sequence) and $31.5 \pm 1.0 \text{ keV}$ (interulses)⁸.

If one compares the limitation on the maximum source luminosity observed in all the recurrent bursts with the Eddington luminosity limit for a neutron star, $I_{\text{Edd}} \sim 10^{38} \text{ erg s}^{-1}$, then from the measured maximum fluence in the recurrent bursts, $I = L_{\text{Edd}}/4\pi d^2 \sim 10^{-6} \text{ erg cm}^{-2} \text{ s}^{-1}$, one can derive an estimate for the distance to the source, $d \sim 1 \text{ kpc}$. Moreover, it is only natural to assume that the recurrent bursts, just as the 5 March event, are generated in the vicinity of the neutron star's magnetic poles. In this case the emitting region will constitute only a small fraction of the total surface of the neutron star, $f \sim 10^{-2}-10^{-3}$, the total source luminosity being determined by the quantity $L_{\text{max}} = fL_{\text{Edd}} \sim 10^{35}-10^{36} \text{ erg s}^{-1}$. Accordingly, the estimate of the distance to the source will become reduced down to $d \sim 30-100 \text{ pc}$.

We conclude that the continuing activity of the GBS0526-66 opens up the possibility of looking for recurrent bursts in other, primarily the soft X-ray and visible spectral regions. If these observations are successful, they may be invaluable for helping to solve one of the most intriguing problems in γ -ray astronomy.

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1. Mazets, E. P. *et al. Nature* **282**, 587-589 (1979).
2. Barat, C. *et al. Astr. Astrophys.* **79**, L24-L26 (1979).
3. Cline, T. L. *et al. Astrophys. J. Lett.* **237**, L1-L5 (1980).
4. Cline, T. L. in *γ -Ray Transients and Related Astrophysical Phenomena*, AIP Conf. Proc. No 77, 17-33 (1982).
5. Mazets, E. P. & Golenetskii, S. V. *Astrophys. Space Sci.* **75**, 47-81 (1981).
6. Golenetskii, S. V. *et al. Pisma Astr. Zh.* **8**, 657-662 (1982).
7. Golenetskii, S. V. *et al. Pisma Astr. Zh.* **5**, 636-640 (1979).
8. Mazets, E. P. *et al. Astrophys. Space Sci.* **84**, 173-189 (1982).
9. Cline, T. L. *et al. Astrophys. J. Lett.* **255**, L45-L48 (1982).

The study of metal-oxide interfaces using sputter ion plating

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The interface between solids has always been difficult to characterize. It is possible to use analytical techniques to probe through an outer surface but matrix effects within the penetrated solid often modify the results to the extent that the interface becomes difficult to detect. The progressive analysis and removal of layers of material from an outer surface until the interface is reached may prove useful but during the removal of thick outer layers the interface becomes indeterminate. A third approach is to dissolve one solid leaving the other intact, but again limitations are encountered. For example, only one side of the interface can be studied and the real boundary may be perturbed or damaged by the dissolving medium. Here we describe a new approach which may be used to study both the metal and oxide side of metal-oxide interfaces. Although this may at first appear to be a rather specialized application, the principles involved may be readily transferred to a number of other systems.

Metal-oxide interfaces may be studied in several ways. For interfaces formed between a relatively thin layer and a bulk material, layers of the thinner entity can be removed by ion sputter-etching the surface. If analysis of the freshly exposed surface is performed simultaneously using Auger electron spectroscopy¹, the surface composition as a function of depth into the specimen may be obtained. This 'depth profiling' capability permits the analysis of a material in three dimensions. When a particular sub-surface feature, such as an interface, is of interest, etching can be stopped when this is reached and a more detailed analysis performed.

A novel technique has been developed by Coad, Tappin and Rivière² for stripping oxide layers from metals to expose the interface on both the oxide and metal side. Previously one of

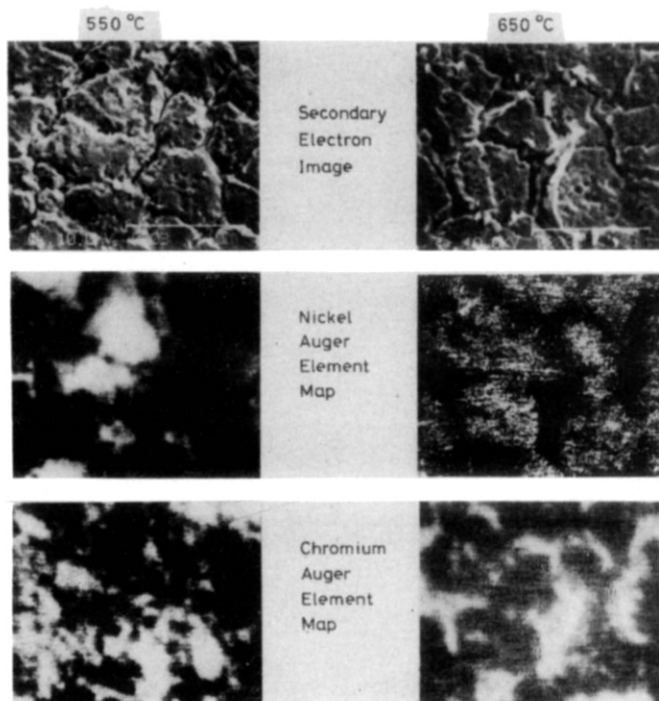


Fig. 1 Scanning electron micrograph and Auger element maps from the surface of a 20% Cr, 25% Ni, Nb stabilized austenitic stainless steel after removal of the oxide layer by sputter ion plating.

the few reliable methods for the removal of an oxide from ferrous materials involved the dissolution of the metal by iodine-methanol solutions. Originally developed by Vernon, Wormwell and Nurse³, this method has been extended more recently to iron-silicon alloys by Moseley, Tappin and Rivière⁴. Although a profile into the oxide could be obtained, the results suffered from the limitation that contamination of the oxide occurred during the process of dissolution. Coad's method, known as sputter ion plating is not subject to this disadvantage because the oxide is simply pulled away from the metal matrix.

The technique involves the deposition of a layer of nickel, or other suitable metal, onto the oxide surface at the elevated temperature of 300 °C. During the deposition process the oxide surface is subjected to bombardment with 1 kV argon ions at a pressure of 30 torr to encourage a strong nickel-oxide bond and the temperature regulated to prevent modification of the oxide film composition. On cooling to room temperature, stresses are induced between the oxide and its nickel overlayer sufficient to pull the oxide away from its base when the coating is deformed. Experiments using sputter ion plating have been carried out on iron-silicon alloys² and in our own laboratory on 20% Cr, 25% Ni, Nb stabilized steel⁵.

The current UK civil advanced gas-cooled nuclear reactor uses a thin-walled 20% Cr, 25% Ni, Nb stabilized austenitic steel cladding for the fuel pins. For heat transfer purposes the surface of the steel tubing is roughened by circumferential ribs of 0.31 mm² section with a pitch of approximately 2.5 mm. Here we report the examination of two samples of this austenitic alloy (total composition 20% Cr, 25% Ni, 0.6% Nb, 0.6% Si, 0.05% C, balance Fe) which had previously been exposed to a CO₂, 1% CO gas mixture at 40 atm for 4,000 h at 550 °C and 650 °C respectively. An oxide layer of thickness between 0.5 and 2.0 μm was formed at the surface. To study the mode of formation and integrity of the oxide, each sample was coated with a 30- μm layer of nickel metal and oxide-metal partition was induced by bending the oxidized cladding alloy parallel to a surface rib. Since separation of the oxide and metal occurred in air, the metal side was contaminated with an air-formed oxide film. However, sputtering experiments using a beam of argon ions of energy 4 keV showed this film to be no more than a few

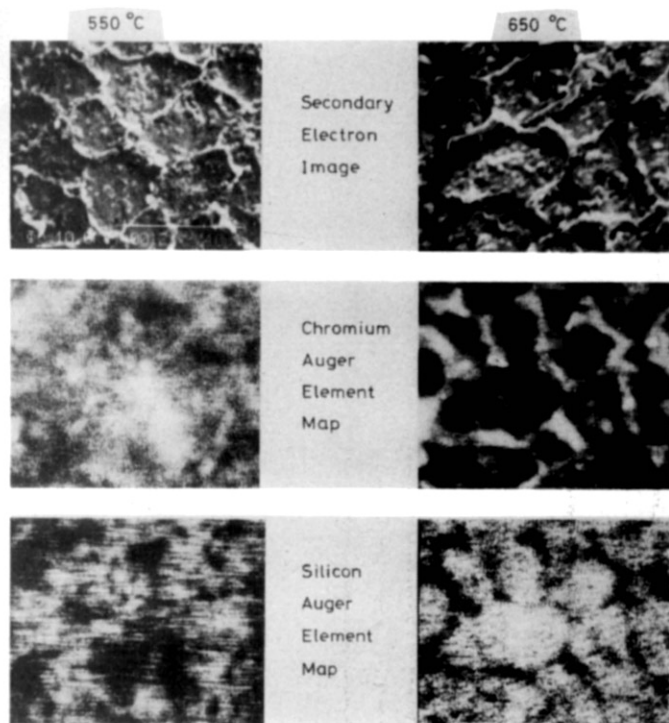


Fig. 2 Scanning electron micrograph and Auger element maps from the oxide side of the metal-oxide interface exposed by sputter ion plating a 20% Cr, 25% Ni, Nb stabilized austenitic steel.

atom layers in thickness and supported the view that separation had occurred at the metal-oxide interface rather than within the oxide.

The alloy side of the oxide-metal interface is illustrated in Fig. 1. It is this surface which cannot be examined readily by other methods. The secondary electron images from the 550 °C and 650 °C samples are very similar. Both show grains of $\sim 20 \mu\text{m}$ diameter within the polycrystalline alloy which are clearly delineated by grooves at the boundary positions. The grooves corresponded to ridges on the oxide side of the interface which were pulled out from the grain boundaries during the process of separation.

Auger electrons emitted by a given element were measured over the area examined in the secondary electron micrograph to provide a visual display of the lateral distribution of the element in question. Maps of this type obtained using the technique of scanning Auger microscopy are illustrated for the elements nickel and chromium beneath the corresponding secondary electron image. Although not illustrated here, results of this kind were also recorded for silicon and iron. As Fig. 1 indicates, striking differences in the distribution of elements were observed from the two samples. At 550 °C an enrichment of nickel at some grain surfaces was noted, together with rather low concentrations at others but, following exposure at 650 °C, a consistently uniform concentration of this element occurred at grain centres with a corresponding depletion at the boundaries of the grains. Similarly, chromium appeared to be randomly distributed across the surface of the metal oxidized at 550 °C but at the higher temperature a significant enrichment was observed in the grain boundary regions. Maps for silicon and iron identified a similar pattern of behaviour, the higher temperature sample revealing the enrichment of silicon at grain boundaries and iron at grain centres.

Scanning electron micrographs of the oxide side of the interface are shown in Fig. 2, together with the Auger element maps from chromium and silicon. Iron and nickel were rarely detected on the oxide surface, an indication that the deposited nickel layer had not penetrated the oxide to the interfacial region. Detailed studies identified a marked difference in the composition of grain surface and grain boundary regions on this side of

the oxide-metal interface. In contrast to the metal, the freshly exposed oxide counterpart of the 650 °C sample showed high levels of silicon at the grain centre and high chromium levels at the grain boundary positions but similar behaviour was not evident in the sample oxidized at 550 °C. Complementary information of this kind can be used to determine the mode of metal-oxide fracture. Results from the experiments at 650 °C, taken together with those from experiments at 850 °C (ref. 6) and argon ion etching, indicated that above the grains at the surface of the metallic structure, separation occurred between the metal crystallites and a very thin layer, $\sim 70 \text{ \AA}$ across, of silicon-rich oxide. At the crystallite boundary regions within the surface of the polycrystalline metal on the other hand, oxide was formed as a wedge down the grain boundary and into the metal so that fracture occurred across the surface and through the bulk of the keyed oxide. Hence the Auger map from the surface, exposed when the oxide was torn away, revealed the presence of chromium, a major constituent of the bulk oxide, in the grain boundary regions.

At 550 °C simple metal-oxide separation no longer occurred. The intervening silicon-rich oxide was incompletely developed across the interfacial region and the metal and oxide were more strongly bound. In fact, the oxide film was so adherent that in certain areas of the surface, separation was only achieved by removing a portion of the metal substrate with the oxide layer.

Providing separation can be achieved, the sputter ion plating technique thus permits the examination of both the metal and oxide sides of a metal-oxide interface by scanning Auger electron spectroscopy. The data so obtained may be used to determine both the mode of separation and the strength of the metal-oxide interaction.

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1. Allen, G. C. & Wild, R. K. *CEGB Res.* **11**, 12-30 (1981).
2. Coad, J. P., Tappin, G. & Rivière, J. C. *AERE Rep.* R10285 (1981).
3. Vernon, W. H. F., Wormwell, F. & Nurse, T. F. *J. Iron Steel Inst.* **150**, 81-92 (1944).
4. Moseley, P. T., Tappin, G. & Rivière, J. C. *AERE Rep.* R10042 (1981).
5. Coad, J. P. & Wild, R. K. *Appl. Surf. Sci.* **14**, 321-329 (1983).
6. Allen, G. C. & Wild, R. K. *Phil. Mag.* **A48**, 373-386 (1983).

Last interglacial reef growth beneath modern reefs in the southern Great Barrier Reef

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Studies of modern coral reefs have shown that Holocene reef growth is relatively thin, and that in many instances it has developed on an older limestone surface¹⁻⁴. This surface is commonly associated with leaching and pedogenic processes, indicative of subaerial exposure, and has been termed the solution unconformity⁵. Investigations into reef growth history have interpreted the pre-Holocene substrate as an older reef surface^{2-4,6-8} that may or may not have undergone karst erosion during periods of lower sea level^{6,9}. Uranium-series dating of corals directly below the uppermost solution unconformity at Eniwetok¹⁰ and Mururoa¹¹ has shown that previous reef growth, before the Holocene, occurred $\sim 120 \text{ kyr}$ ago. We present here ²³⁰Th/²³⁴U dates that confirm a last interglacial age for reefal limestones directly beneath the uppermost solution unconformity in the southern Great Barrier Reef.

Previous drilling on the Great Barrier Reef has encountered the topmost solution unconformity at depths ranging from 4 to 22 m below sea level²⁻⁴. Radiocarbon dating of material below the unconformity has produced ages that are beyond the detection limit of this method, and there has been insufficient suitable

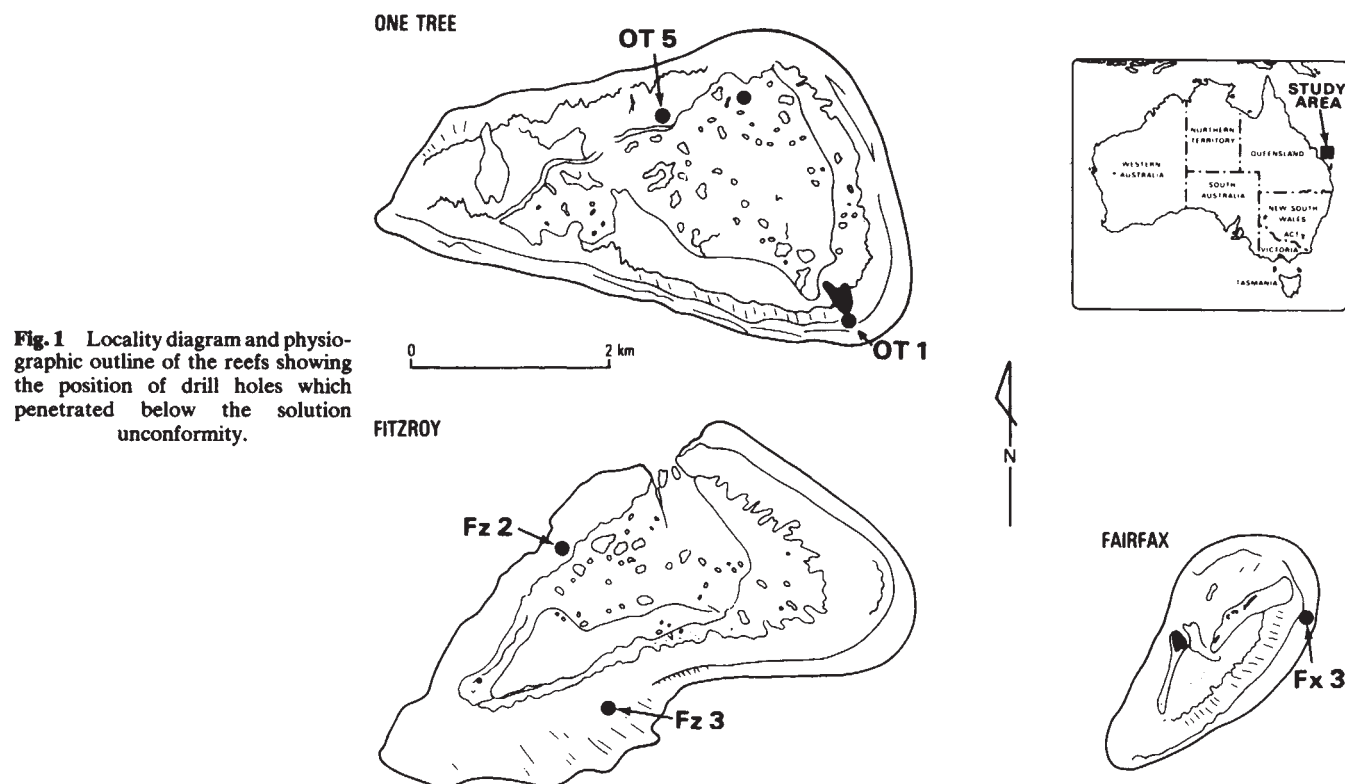


Fig. 1 Locality diagram and physiographic outline of the reefs showing the position of drill holes which penetrated below the solution unconformity.

material to enable absolute age determinations using the uranium-series technique³. Since then, shallow drilling operations in the southern Great Barrier Reef have penetrated the solution unconformity at depths ranging from 7.4 to 14.3 m on three reefs (Figs 1 and 2). Previously, seismic refraction profiling on these reefs indicated the solution unconformity at depths ranging from 8 to 23 m (ref. 12). Radiocarbon dating of corals above the unconformity have confirmed that it is Holocene¹³⁻¹⁵, whereas below it, unrecrystallized corals produced radiocarbon ages in excess of 30,000 yr BP (ref. 14). Core material below the unconformity is commonly leached and cemented by low-Mg calcite, while in some cores the limestone is impregnated with soil humates or contains cross-cutting stringers of brown calcrete containing numerous rhizocretions. However, while the material below the unconformity shows the effects of subaerial vadose diagenesis, aragonite corals suitable for dating were found in five of the drill holes (Fig. 2).

The results of $^{230}\text{Th}/^{234}\text{U}$ dating of these corals are shown in Table 1. All the corals obey the closed system criteria, and their uranium contents and $^{234}\text{U}/^{238}\text{U}$ ratios fall within the expected ranges.

Two anomalous ages of 107 kyr from Fairfax Reef (hole 3) and 172 kyr from Fitzroy Reef (hole 2) are considered to have resulted from these corals not being in their original growth position. The 107-kyr coral from Fairfax Reef occurs directly

on top of a pronounced soil horizon that represents the surface of the solution unconformity. Previously, this coral had produced a minimum ^{14}C age of 30,000 yr BP. Its presence above the unconformity suggests that it is not *in situ*. The 172-kyr age from Fitzroy Reef (hole 2) is more difficult to explain. In the absence of age determinations both above and below it, no reliance is placed on its accuracy.

Most corals occur in the age range of 122–145 kyr. This spread in the ages is consistent with results from previously dated last interglacial terraces¹⁶⁻¹⁹ and the submerged foundations of atolls^{10,11}. The two ages from One Tree Reef are older than those from Fairfax and Fitzroy Reef (hole 3). This could reflect variations in the timing of reef growth and their respective growth rates, such as seen in the modern reef. However, variations of this type in the Holocene reefs are only of the order of 2,000–4,000 yr, whereas the difference between One Tree and the other reefs is >10,000 yr. Alternatively, it could reflect differing rates of subaerial erosion of the last interglacial reefs during the period of low sea level before the last transgression—a period of some 100,000 yr. The relatively greater depths to the solution unconformity in the One Tree Reef drillholes (Fig. 2) would seem to bear out this latter alternative.

On the assumption that sea level during the last interglacial was ~5 m higher than present, and that the reefs were capable of attaining this high sea level elevation, the present position of

Table 1 Radiochemistry and ages of coral samples beneath the solution unconformity in the southern Great Barrier Reef

Drill hole	Sample no.	Depth (m)	Mineralogy (% aragonite)	U (p.p.m.)	$^{234}\text{U}/^{238}\text{U}$	$^{230}\text{Th}/^{234}\text{U}$	Age (kyr)
One Tree Reef							
1	78632219	13.4–13.7	100	3.82	1.15 ± 0.01	0.75 ± 0.02	145 ± 8
5	79632597	15.0–16.7	95	3.38	1.10 ± 0.01	0.73 ± 0.03	138 ± 11
Fairfax Reef							
3	79632955	8.5–8.8	100	2.78	1.14 ± 0.03	0.64 ± 0.02	107 ± 8
3	79632967	11.1–11.2	100	4.03	1.08 ± 0.01	0.70 ± 0.02	127 ± 8
Fitzroy Reef							
2	79632734	8.7–8.9	98	3.67	1.11 ± 0.01	0.81 ± 0.02	172 ± 12
3	79632838	10.6–11.0	100	2.57	1.10 ± 0.01	0.69 ± 0.02	125 ± 6
3	79632856	13.0–13.2	100	3.51	1.10 ± 0.01	0.68 ± 0.02	122 ± 6

$^{232}\text{Th}/^{230}\text{Th} < 0.05$ for all samples. Errors shown are based on counting statistics $\pm 1\sigma$.

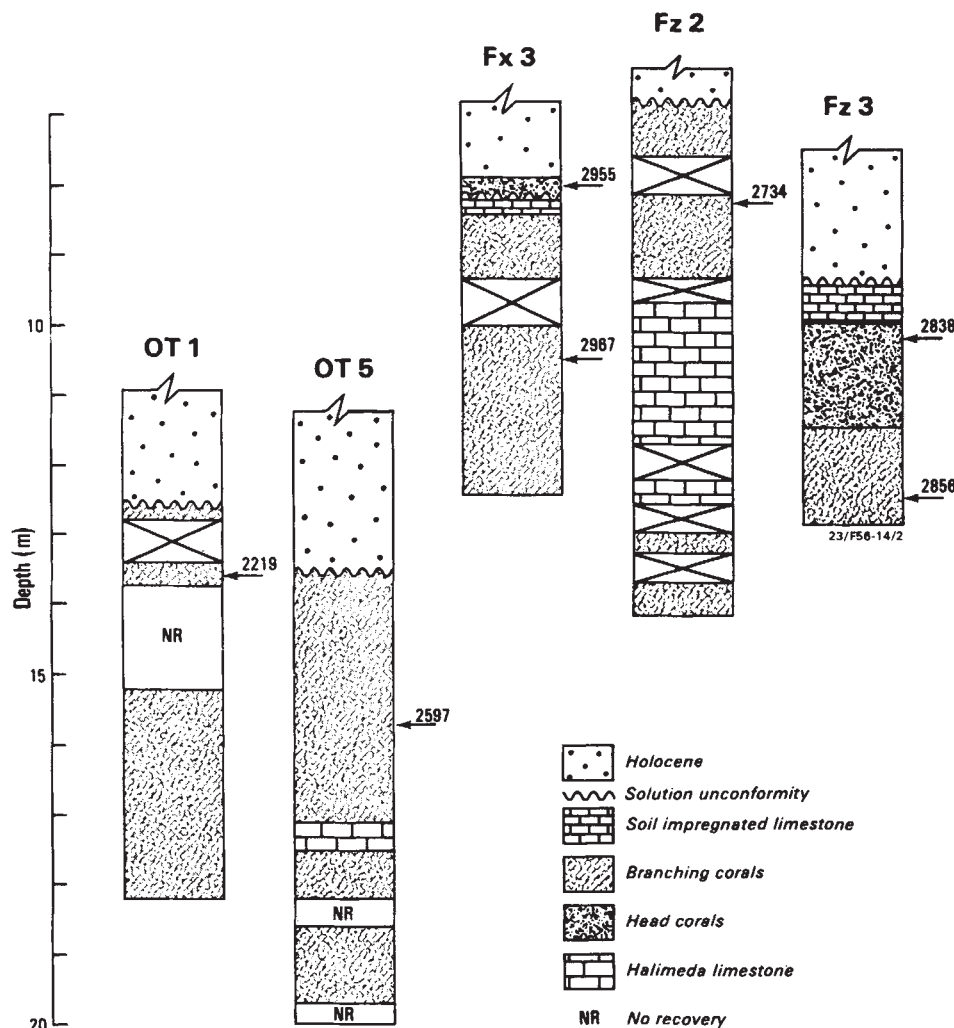


Fig. 2 Lithological logs of the pre-Holocene sections of the drill holes showing the position of the dated coral samples.

the solution unconformity in the drillholes suggests a lowering of the reef's surface of the order of 12–19 m during the intervening period between the last interglacial and the Holocene transgression. The position of last interglacial reefs and other strand-line features removed from plate boundaries have verified that the sea level was higher than present by ~4–6 m (refs 19, 20). On the basis of present-day and Holocene growth rates it would have been possible for all the reefs dated here to have reached this high sea level position within the period of the last interglacial. For example, the 127-kyr date at ~11 m in hole 3 at Fairfax Reef (Table 1) indicates that for a uniform vertical accretion rate of 4 m kyr^{-1} , this section of the reef would have reached the position of the last interglacial high sea level by 123 kyr ago. Providing that the subsidence rate of the continental margin (0.05 m kyr^{-1}) has remained uniform²¹, the present position of the unconformity would require a rate of erosion of the reefs' surface of the order of $0.07\text{--}0.14 \text{ mm yr}^{-1}$, a rate that is not unrealistic for subaerial weathering of reefal limestones²². Differential erosion between the perimeters of the reefs and their lagoons, possibly as a result of submarine lithification within the former, would accentuate the original relief in such a manner as to lend credence to the antecedent karst hypothesis⁶.

The determination of the exact age and depth of the modern reef substrate has long been considered as crucial to understanding the development of coral reefs. The arguments regarding the age and nature of the substrate go back as far as Darwin's²³ and Daly's²⁴ theories on reef development, and continue to the present day. Before our results there were only two reefs (Eniwetok and Mururoa) where it had been shown that the foundations of extant Holocene reefs were last interglacial in age. Because these are both atolls, it is encouraging to find a

similar age in a different tectonic setting, such as the shelf reefs of the Great Barrier Reef.

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1. Adey, W. H. *Atoll Res. Bull.* **187**, 1–67 (1975).
2. Thom, B. G., Orme, S. R. & Polach, H. A. *Phil. Trans. R. Soc. A* **291**, 37–54 (1978).
3. Hopley, D., McLean, R. F., Marshall, J. & Smith, A. S. *Search* **9**, 323–325 (1978).
4. Davies, P. J. & Marshall, J. F. *Search* **10**, 276–279 (1979).
5. Schlanger, S. O. *U.S. Geol. Surv. Prof. Pap.* **160-BB**, 991–1066 (1963).
6. Purdy, E. G. in *Reefs in Time and Space* (ed. Laporte, L. F.) 9–76 (Sepm Spec. Publ. 18, 1974).
7. Halley, R. B., Shinn, E. A., Hudson, J. F. & Lidz, B. *Proc. 3rd int. Coral Reef Symp.* **2**, 29–35 (1977).
8. Emery, K. O., Tracey, J. I. & Ladd, H. A. *U.S. Geol. Surv. Prof. Pap.* **260-A**, 1–265 (1954).
9. Adey, W. H. *Science* **202**, 831–837 (1978).
10. Thurber, D. I., Broecker, W. S., Blanchard, R. L. & Potratz, H. A. *Science* **149**, 55–58 (1965).
11. Lalou, C., Labeyrie, J. & Delibrias, G. *C. r. heb. Séanc. Acad. Sci., Paris D* **263**, 1946–1949 (1966).
12. Harvey, N., Davies, P. J. & Marshall, J. F. *BMRI Aust. Geol. Geophys.* **4**, 141–147 (1979).
13. Davies, P. J. & Marshall, J. F. *Nature* **287**, 37–38 (1980).
14. Marshall, J. F. & Davies, P. J. *Coral Reefs* **1**, 21–28 (1982).
15. Davies, P. J. & Marshall, J. F. *Coral Reefs* (in the press).
16. Broecker, W. S. *et al. Science* **159**, 297–300 (1968).
17. Bloom, A. L., Broecker, W. S., Chappell, J. M. A., Matthews, R. K. & Mesolella, K. J. *Quat. Res.* **4**, 185–205 (1974).
18. Chappell, J. & Veeh, H. H. *Bull. geol. Soc. Am.* **89**, 356–368 (1978).
19. Ku, T. L., Kimmel, M. A., Eastorn, W. H. & O'Neil, T. J. *Science* **185**, 959–962 (1974).
20. Marshall, J. F. & Thom, B. G. *Nature* **263**, 120–121 (1976).
21. Davies, P. J. in *Perspectives on Coral Reefs* (ed. Barnes, D. J.) 69–106 (Brian Clouston, Canberra, 1983).
22. Trudgill, S. T. Z. *Geomorphol.* **26**, 201–210 (1976).
23. Darwin, C. R. *The Structure and Distribution of Coral Reefs* (Smith Elder, London, 1842).
24. Daly, R. A. *Proc. Am. Acad. Arts Sci.* **51**, 155–251 (1915).

Late Quaternary pollen records from Easter Island

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Easter Island is the most isolated piece of inhabited land in the world. It exhibited an unique megalithic culture¹⁻³, involving the sculpting of giant statues (moai) especially between AD ~1400 and ~1680⁴ when, for unknown reasons, the culture suddenly collapsed. The island is also of interest in relation to Pleistocene climatic change as CLIMAP⁵ predicted no reduction of sea-surface temperatures for this part of the Pacific at 18,000 yr BP. We have obtained fossil pollen records covering the past 37,000 yr from three craters on the island. They suggest that the late Pleistocene climate was cooler and/or drier than the present one. They also suggest the former existence of forest on the island, and its decline in the last millennium. This decline was probably due to deforestation by man and could have caused the cultural collapse.

The island (lat. 27°9' S; long. 109°26' W) appears to have been substantially without woody vegetation since its discovery

in 1722⁶⁻⁸. The present vegetation is mostly grassland. The known native flora consists of 46 vascular species⁹ of which only one, the endemic *Sophora toromiro* (Phil.) Skottsb., is a tree. This species is now extinct on the island, but survives in botanical gardens¹⁰. The only native shrubs are *Lycium carolinianum* Walt. and *Caesalpinia bonduc* (L.) Roxb. The chief herbaceous families represented are the Gramineae and Cyperaceae.

Initial work on the Quaternary palynology of Easter Island, without dating, was carried out by Selling⁴ who showed that a woody vegetation had formerly occurred. He found pollen of a palm, which he believed might be *Pritchardia* sp., now extinct on the island.

In the present study, the deposits of three volcanic craters were cored, and single complete cores returned to the laboratory. All three craters hold freshwater, and the deposits are highly organic. The stratigraphy has been described elsewhere¹¹, and appears to be relatively simple. ¹⁴C and ²¹⁰Pb dating were carried out on samples from the pollen cores. Figures 1-3 outline pollen diagrams; full results will be published later.

Oceanic islands are well known as recipients of pollen from distant sources¹². In the present work some sparsely represented taxa, such as *Ephedra* and *Casuarina*, are thought to have arrived in this way, but the major pollen contributors, discussed here, are presumed to have grown on the island.

The dating results require comment. The Rano Raraku ¹⁴C dates (Fig. 1) are augmented by assay for ²¹⁰Pb of 33 samples from the top 70 cm. The results suggest a mean accumulation

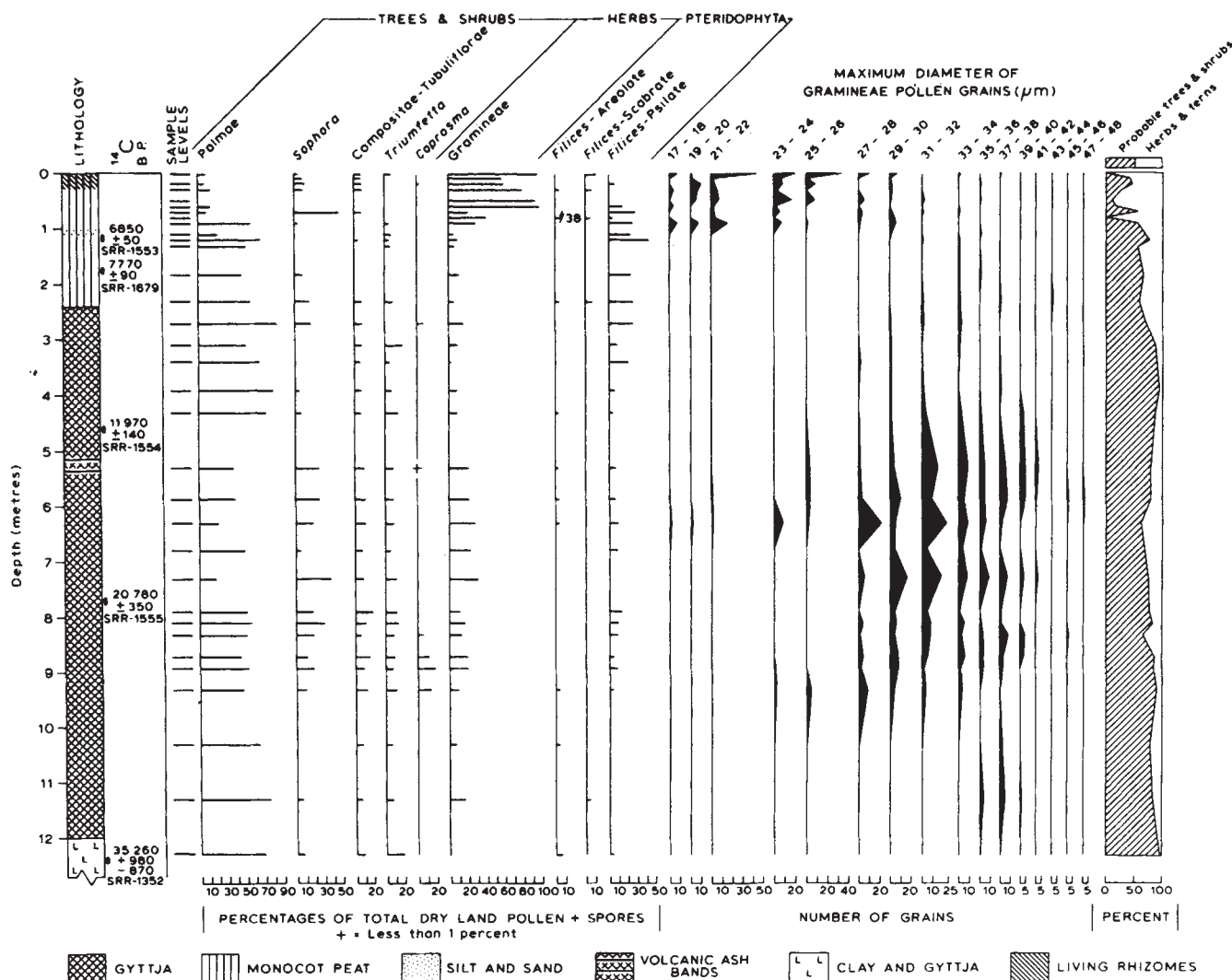


Fig. 1 Pollen diagram from Rano Raraku, alt. ~75 m. Only selected taxa are shown. Unidentifiable and unidentified grains only once represented more than 5% of the total. The summary diagram at right shows percentages of total dry land pollen and spores, not just of the taxa illustrated.

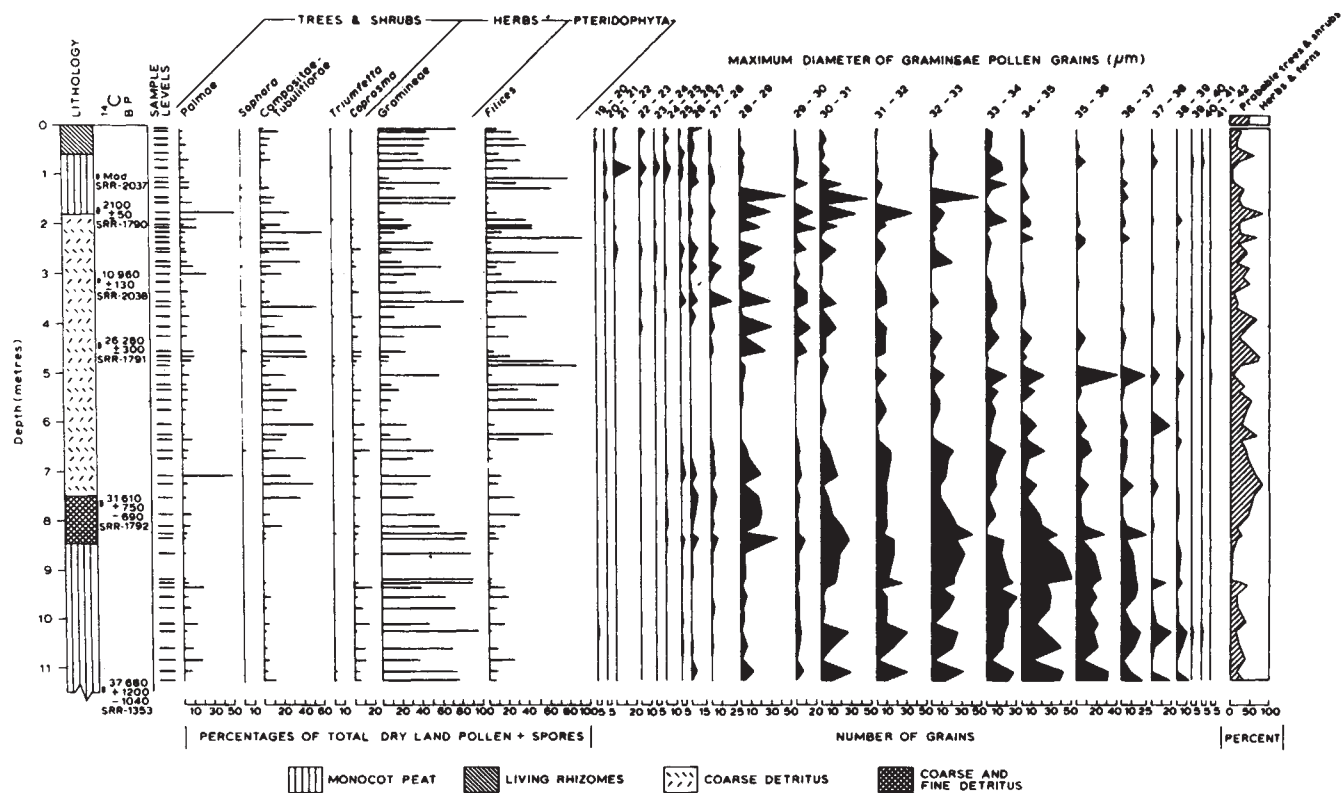


Fig. 2 Pollen diagram from Rano Aroi, alt. ~425 m. Only selected taxa are shown. Unidentifiable and unidentified grains never represented more than 2% of the total. The summary diagram at right shows percentages of total dry land pollen and spores, not just of the taxa illustrated.

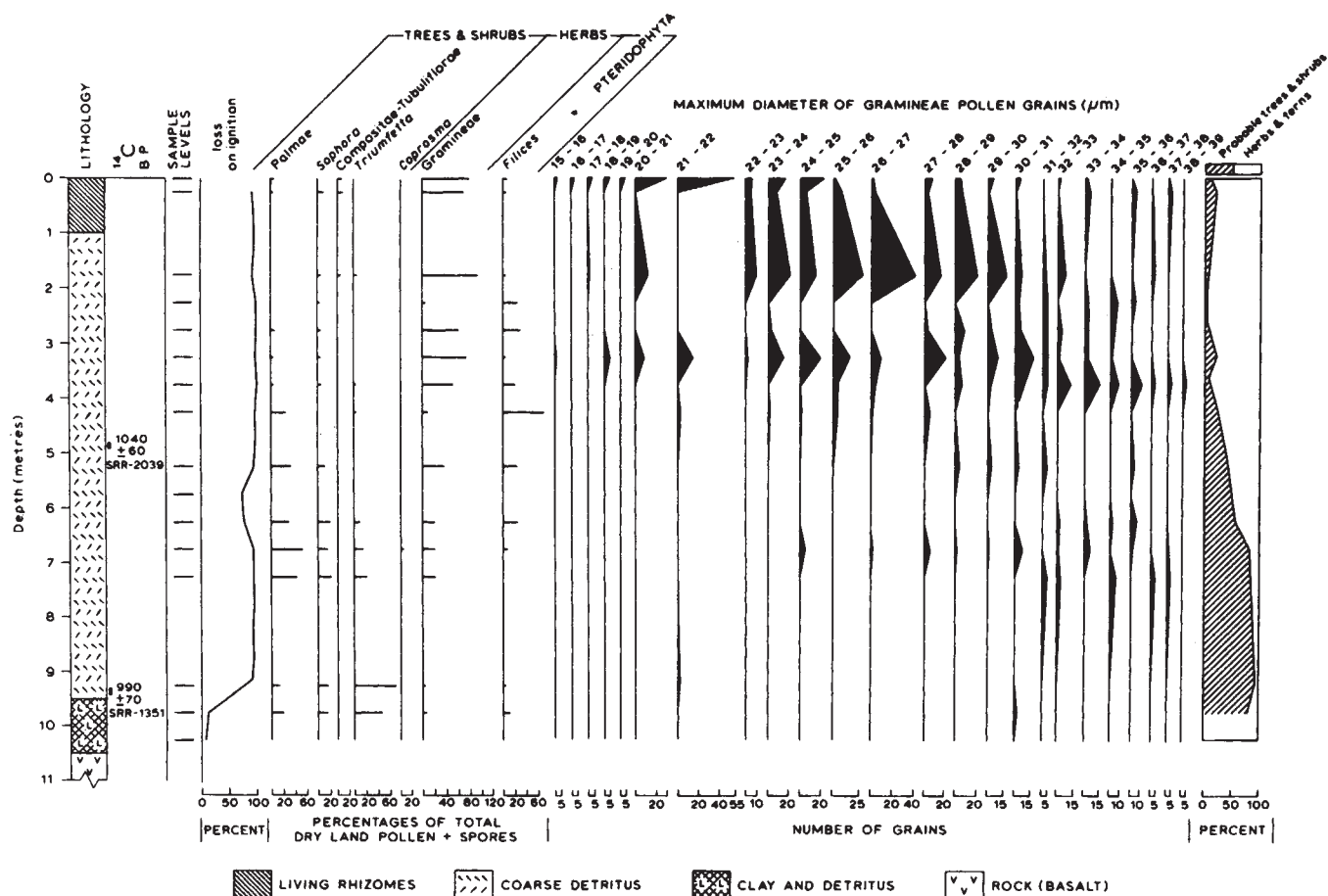


Fig. 3 Pollen diagram from Rano Kao, alt. ~110 m. Only selected taxa are shown. Note the changes of scale. Unidentifiable and unidentified grains were never more than 5% of the total. The summary diagram at right shows percentages of total dry land pollen and spores, not just of the taxa illustrated.

Years BP												
		0	500	1000	10 000		21 000		26 000	32 500	35 500	37 500
RANO RARAKU	VEGETATION	Grassland		Palm forest with <i>Sophora</i> and shrubs			Palm and <i>Sophora</i> woodland with Gramineae		Palm forest with <i>Sophora</i> and shrubs			
	CLIMATE	Present		Not necessarily different from present			Cooler and/or drier than at present		Not necessarily different from present			
	HUMAN DISTURBANCE	Complete deforestation		Nil or slight			Nil		Nil			
RANO AROI	VEGETATION	Grass/Ferns		Shrubs and ferns, some grasses			Grasses and shrubs, some ferns			Shrubs and ferns some grasses		Chiefly Grass-land
	CLIMATE	Present		Not necessarily different from present			Cooler and or drier than at present			Not necessarily different from present		Cooler and/or drier than at present
	HUMAN DISTURBANCE	Removal of shrubs		Nil or slight			Nil			Nil		Nil
RANO KAO	VEGETATION	Grass-land	Mixed forest									
	CLIMATE	Present	Present									
	HUMAN DISTURBANCE	Complete deforestation	Slight ?									
AREAS OF SELF CONSISTENCY	VEGETATION	Grass-land		Woody plants present				Altitudinal limit of woody vegetation below 425m		Woody plants present		
	CLIMATE	Present		Not necessarily different from present				Cooler and/or drier than at present		Not necessarily different from present		
	HUMAN DISTURBANCE	Complete deforestation		Nil or slight				Nil		Nil		

Fig. 4 Tentative conclusions as to former vegetation, climate and human disturbance on Easter Island. The time scale is given as a square root transformation.

rate of $\sim 1 \text{ cm yr}^{-1}$ for this part of the deposit. A date of 70 BP for 70-cm depth accords with the lower three dates in the core if a steady rate of accumulation is assumed below 70 cm, but this suggests that SRR-1553 and SRR-1679 are too old. This is feasible because they are close to the layer of inwashed silt and sand at 1.10 m. This probably indicates the inwash of soil carbon, a well known cause of contamination in radiocarbon dating¹³. The linear accumulation rate is therefore preferred for dating purposes. At Rano Kao (Fig. 3), similar contamination is suggested by the drop in loss-on-ignition values at ~ 6 -m depth, and SRR-2039 is consequently rejected. At Rano Aroi (Fig. 2) there seem to have been changes in the rate of deposition. SRR-1790 is stratigraphically close to inwashed material seen in another borehole, and it could therefore be too old.

Figures 1–3 show evidence of the occurrence on the island of taxa which are now extinct there. The palm pollen found by Selling is abundant at all three sites. *Pritchardia*, which occurs on many Polynesian islands¹⁴, has very similar pollen¹⁵, but other possibilities cannot be excluded on pollen morphological grounds. This pollen taxon has been included in 'probable trees and shrubs'. There is also abundant pollen of Compositae, subfamily Tubuliflorae. Pollen morphology places this taxon in the tribe Heliantheae¹⁶. Plant geographical considerations make the most likely taxa *Chrysogonum* and *Campylothea*^{14–17}, both of which comprise trees or shrubs. This pollen taxon has therefore also been included in 'probable trees and shrubs'.

The pollen records do not include all the taxa believed to be native today. Note that the two shrubs, *C. bonduc* and *L. carolinianum* are absent, but as they are both entomophilous, their absence is not particularly surprising. A total of 40 pollen taxa were recorded.

It is theoretically possible that some of the changes seen in the pollen record might be related to changes of facies in the stratigraphy. Thus at Rano Aroi the abundance of Tubuliflorae coincides with the occurrence of coarse detritus. The deposit contains no macroscopic remains of Tubuliflorae, however, which might have been expected if Tubuliflorae had been growing on the swamp. At Rano Raraku the rise of Palmae coincides with the occurrence of two 1-cm thick bands of volcanic ash at

~ 5 -m depth, but it seems unlikely that so minor a volcanic event could have caused a vegetational change lasting several thousand years. The effect of such facies changes is therefore discounted.

For conciseness the vegetational and climatic interpretations will be considered simultaneously. To do this it is necessary to consider now the climatic significance of the major pollen taxa. *Sophora* grew on the island until recently, when it was probably destroyed by felling and grazing, and it may be taken as suggesting a climate similar to the present one. Palmae and Tubuliflorae were also present until recently (according to the pollen record), but Palmae were more abundant at Rano Raraku (~ 75 -m altitude) and Tubuliflorae at Rano Aroi (~ 425 -m altitude). This suggests that Palmae are indicative of warmer temperatures and Tubuliflorae of cooler temperatures, within the range found on the island today.

The Gramineae pollen of the record falls into two main categories. Grains below $\sim 27 \mu\text{m}$ maximum diameter dominate the recent part of the record and are believed to be chiefly those of the grasses, both introduced and native, which now dominate most parts of the island. Grains above this size occur chiefly in the earlier part of the record and are thought to represent other native grass species which were more frequent before disturbance. They are particularly abundant in the Rano Aroi record and are probably therefore to be associated with the lower temperatures within the range found on the island.

It is certainly possible that both Gramineae and Palmae would have been favoured by drier conditions on the island in the past, as judged by the known ecological preferences of these families, but we have as yet no information on this point in relation to Easter Island taxa.

Bearing all these considerations in mind and interpolating where necessary between dates, it is possible to draw the tentative conclusions as to former vegetation, climate and human disturbance shown in Fig. 4. These will have to be revised as further dates and pollen and chemical analyses become available. Already, however, there emerges a fairly coherent picture. The chief conclusions are: (1) the island was probably formerly forested; (2) deforestation occurred, probably over a

considerable time span, but since 990 ± 70 yr BP at Rano Kao; (3) the climate was cooler and/or drier than at present during the period ~21,000–12,000 yr BP.

The chief interest arising from conclusion (3) is the general correlation in time and direction with late Pleistocene climatic changes elsewhere in both hemispheres, and the possible contrast with CLIMAP conclusions⁵. It is hoped that further work will clarify and quantify the climatic shift involved. Conclusions (1) and (2) are of archaeological interest, and do not conflict with the hypothesis¹⁸ that the decline of the megalithic culture was associated with total deforestation. The latter could have been effective through shortage of food resulting from non-availability of fertile forest soils. Alternatively, or additionally, large timbers may have been essential for the moving of the moai^{18,19} and deforestation could, therefore, have made it pointless to continue to carve them.

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1. Routledge, K. *The Mystery of Easter Island: The Story of an Expedition* 165–199 (Sifton, Praed, London, 1919).
2. Mettraux, A. *Easter Island: A Stone-Age Civilization of the Pacific* pp. 149–170 (Andre Deutsch, London, 1957).
3. Mettraux, A. *Bernice P. Bishop Mus. Bull.* No. 160 (1971).
4. Heyerdahl, T. & Ferdon, E. N. Jr (eds) *Reports of the Norwegian Archaeological Expedition to Easter Island and the East Pacific* Vol. 1 (Forum, Stockholm, 1961).
5. CLIMAP, *Science* **191**, 1131–1137 (1976).
6. Gonzalez, D. F. *The Voyage of Captain Don Felipe Gonzalez in the Ship of the Line San Lorenzo, with the Frigate Santa Rosalia in Company, to Easter Island in 1770–01* (Hakluyt Society, Cambridge, 1908).
7. Cook, J. A. *Voyage towards the South Pole and Round the World, 1772–75*, 1 (Strahan & Cadell, London, 1777).
8. La Perouse, J. F. de G. *Voyage de La Perouse Autour du Monde*, Paris, 1797 (English translation) (Johnson, London, 1798).
9. Skottsborg, C. *The Natural History of Juan Fernandez and Easter Island* Vol. 1, 193–438 (Almqvist & Wiksells, Uppsala, 1956).
10. Alden, B. *Nouveau Regard sur L'île de Paques*, 119–122 (Moana, Corbeil, 1982).
11. Flenley, J. R. *Asian Perspect.* **22**, 33–40 (1979).
12. Hafsten, U. *Arbok Univ. Bergen, Mat-Naturv.* Ser. 20, 1–48 (1960).
13. Pennington, W., Cambray, R. S., Eakins, J. D. & Harkness, D. D. *Freshwater. Biol.* **6**, 317–331 (1976).
14. Balgooy, M. M. J. van *Plant Geography of the Pacific, Blumea Suppl.* **6**, 1–222 (1971).
15. Selling, O. H. *Bernice P. Bishop Mus. Spec. Publ.* 37–39, Honolulu (1946–48).
16. Salgado-Labouriau, M. L. *Grana* **21**, 97–102 (1982).
17. Brown, F. B. H. *Bernice P. Bishop Mus. Bull.* No. 130 (1935).
18. Mulloy, W. J. *Archaeol. phys. Anthropol. Oceania* **5**, 1–23 (1970).
19. Heyerdahl, T. *Aku-Aku* (Allen and Unwin, London, 1958).

A resetting of Phanerozoic community evolution

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The early part of an adaptive radiation is thought¹ to show more taxonomic turnover than later stages. I show here that the effect on probability of extinction is one of exponential decrease, and that in the case of marine families this regular decline was interrupted by the great Permian extinction. A new exponential decrease followed, at a somewhat faster rate. The Cretaceous–Palaeogene extinction is the only other one detectably different from the scatter, and it had no apparent effect on extinction in the Cenozoic. These regularities support an interactive view of large-scale community evolution but do not uniquely determine the nature of the interaction.

Sepkoski² has critically compiled the known geological ranges, by stage whenever possible, of all marine families of invertebrates and vertebrates. I used his summary for the families which are readily preservable in the fossil record.

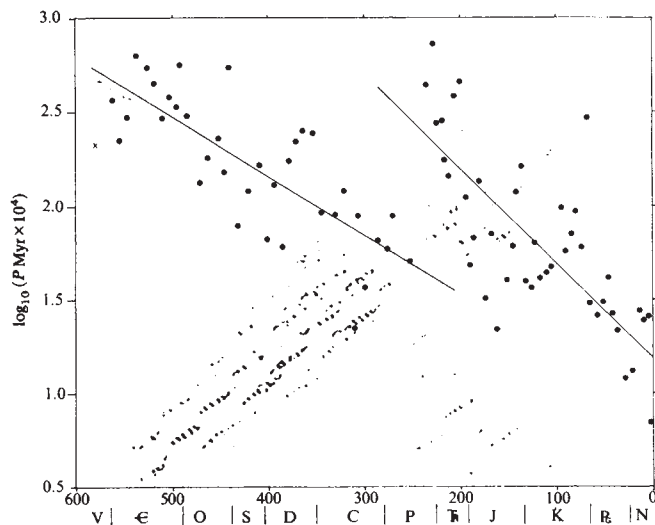


Fig. 1 Probabilities of extinction per Myr (P_{Myr}) through geological time, on a semilogarithmic scale. The abscissa is scaled in Myr BC and by standard symbols for geological periods (Table 1). The plotted regressions exclude the Vendian, because the datum plotted (x) includes soft-bodied forms, and the Maestrichtian, because of its uniquely divergent position. For the left regression, through the Leonardian, $y = -0.00313x + 0.904$, $r = 0.775$, $s_{yx} = 0.235$. For the right regression, from the Guadalupian, $y = -0.00502x + 1.191$, $r = 0.800$, $s_{yx} = 0.275$. (If the Maestrichtian is included, $y = -0.00478x + 1.240$, $r = 0.749$, $s_{yx} = 0.308$.)

My analysis is based on probability of extinction³ rather than absolute numbers of extinctions, the more common procedure. These approaches give different results when the total numbers of taxa differ among the set of taxa in a comparison. An epistemic (or a subjective or propensity) interpretation of probability gives a meaning to the probability of a single event like an extinction, unlike the standard frequentist interpretation of probability. Probability of extinction is appropriate both for single taxa and for communities or broader groups; the same number of extinctions has a smaller expected effect when more taxa are present, just as for the same number of deaths in differently sized populations of individuals.

Probability of extinction decreased rather regularly throughout the Palaeozoic (Fig. 1). However, the late Permian extinction was somewhat larger than even those of the Cambrian, and even in the early and middle Triassic the probability was still about that for the Ordovician. Thus a new decrease was initiated, which has continued to the present. All but two of the post-Palaeozoic probabilities lie above an extension of the Palaeozoic regression. The scatter is homogeneous above and below the regressions and between the two regressions, and normally distributed about the regressions, but the later regression is significantly ($P < 0.02$, $P < 0.05$ if Maestrichtian included) steeper than the earlier one.

Other than the late Permian extinction with respect to the Palaeozoic regression, only the Maestrichtian (late Cretaceous) extinction is more than two standard deviations above the appropriate regression. This does not mean that all other extinctions are causally homogeneous, but any heterogeneity cannot be detected from this sort of analysis (*contra* Raup and Sepkoski⁴). It is therefore not feasible to estimate frequencies of collisions with large meteoroids, as has been done¹².

Some aspects are better seen in Fig. 2. The high Cambrian probabilities were preceded by a rise, ostensibly from no extinction at all in the Vendian. (The probability including soft-bodied forms is artificially high because such forms are almost never found in the early Cambrian; its true value could even be zero also.) A clustering of high probabilities occurs in the early Cambrian, late Devonian, late Permian, late Triassic and late

Table 1 Probabilities of extinction (ext.), by stage or the equivalent, of normally fossilizable marine families

Stage	P (ext. per stage)	P (ext. per Myr)	Stage	P (ext. per stage)	p (ext. per Myr)
Middle and upper Vendian (V)			Triassic (Tr)		
Norm. preserved	0	0	Induan	0.0831	0.0277
All	0.591	0.0215	Olenekian	0.0977	0.0279
Cambrian (C)			Anisian	0.0628	0.0179
Early Tommotian	0.218	0.0363	Ladinian	0.0802	0.0146
Late Tommotian	0.157	0.0224	Carnian	0.1472	0.0391
Atdanian	0.265	0.0294	Norian	0.2058	0.0457
Botomian	0.813	0.0625	Rhaetian	0.0781	0.0112
Lenian	0.373	0.0533	Jurassic (J)		
Early middle Cambrian	0.317	0.0453	Hettangian	0.0148	0.0049
Late middle Cambrian	0.326	0.0296	Sinemurian	0.0407	0.0068
Early late Cambrian	0.230	0.0383	Pliensbachian	0.0677	0.0135
Middle late Cambrian	0.167	0.0334	Toarcian	0.0227	0.0032
Late late Cambrian	0.224	0.0560	Bajocian	0.0418	0.0070
Ordovician (O)			Bathonian	0.0205	0.0022
Tremadocian	0.331	0.0301	Callovia	0.0278	0.0040
Arenigian	0.160	0.0133	Oxfordian	0.0363	0.0061
Llanvirnian	0.183	0.0183	Kimmeridgian	0.0356	0.0119
Llandeilan	0.116	0.0232	Tithonian	0.0814	0.0163
Caradocian	0.137	0.0152	Cretaceous (K)		
Ashgillian	0.267	0.0534	Berriasian	0.0159	0.0040
Silurian (S)			Valanginian	0.0183	0.0037
Llandoveryan	0.0945	0.0079	Hauterivian	0.0326	0.0065
Wenlockian	0.1315	0.0120	Barremian	0.0249	0.0042
Ludlovian	0.1632	0.0163	Aptian	0.0315	0.0045
Devonian (D)			Albian	0.0384	0.0048
Gedinnian	0.0602	0.0067	Cenomanian	0.0792	0.0099
Siegenian	0.0654	0.0131	Turonian	0.0346	0.0058
Emsian	0.0548	0.0061	Coniacian	0.0289	0.0092
Eifelian	0.1204	0.0172	Santonian	0.0377	0.0094
Givetian	0.1534	0.0219	Campanian	0.0491	0.0061
Frasnian	0.2015	0.0252	Maestrichtian	0.1492	0.0298
Famennian	0.2427	0.0243	Palaeogene (PG)		
Carboniferous (C)			Danian	0.0154	0.0031
Tournaisian	0.1157	0.0093	Middle and late Palaeocene	0.0172	0.0026
Viséan	0.1111	0.0089	Early Eocene	0.0140	0.0031
Namurian	0.0981	0.0123	Middle Eocene	0.0205	0.0041
Bashkirian	0.0224	0.0022	Late Eocene	0.0192	0.0027
Moscovian	0.0539	0.0037	Early and middle Oligocene	0.0109	0.0022
Stephanian	0.0816	0.0065	Late Oligocene	0.0094	0.0012
Permian (P)			Neogene (N)		
Asselian	0.0383	0.0059	Early Miocene	0.0118	0.0013
Sakmarian	0.0616	0.0088	Middle Miocene	0.0128	0.0028
Leonardian	0.1320	0.0050	Late Miocene	0.0140	0.0025
Guadalupian	0.4423	0.0442	Pliocene	0.0082	0.0026
Dzhulfian	0.3606	0.0721	Pleistocene	0.0013	0.0007

Each probability was estimated as the number of extinctions (last appearances) in the stage, divided by the average number of taxa at risk. The latter was estimated as the number of taxa at the beginning of the interval, minus half the extinctions, plus half the originations (first appearances) in the interval. This assumes that extinctions and originations are evenly distributed in the interval, which is not always true and can be easily corrected for when so desired, but even when the assumption is untrue the method provides an average value for the whole stage. For the middle and late Vendian this procedure is inappropriate because of the rapidly increasing diversity with few extinctions likely within the period; I estimated the taxa at risk as the number of extinctions plus half the number of survivors and used half the duration of the period as the time at risk. Probability per stage is appropriate if extinctions are concentrated at the end of stages. Questionable records were ignored, and the data include vertebrates as well as invertebrates. Data with accuracy less a stage were divided equally among stages, a procedure which undoubtedly makes at least the Cretaceous-Palaeogene extinction seem somewhat less extreme than it really was. Standard errors of the probabilities cannot be determined but are certainly less than the standard deviations about the two regressions in Fig. 1. Pseudoextinctions are negligible; in fact, I can think of no real case of pseudoextinction in the fossil record at the family level, which would require the last surviving species of an earlier family to give rise to the next family.

Jurassic, with some suggestions of rising probabilities before most of these local maxima.

The 'Great Dying' at the end of the Cretaceous would be unusually low for the Cambrian, and the late Devonian extinction (including more than just the Frasnian, *contra* the usual view) would be ordinary for the Ordovician. Cambrian (biomere) extinctions as well as that of the Cretaceous were mostly concentrated at the ends of stages, so probabilities per stage rather than per time are a better measure (Table 1). Apart from the Maestrichtian, the three largest deviations from the regressions are below rather than above. If these and similar deviations are real rather than artefacts of inaccuracy of the data (including stage lengths), they deserve study for their own sake. What causes intervals of low extinction? Such an interval, like one of stasis, is a real datum.

Darwin⁵ and many others have proposed that organisms increase their absolute fitnesses, the average increasing through geological time. However, this does not appear to explain the pattern of Fig. 1 because there are two declines in probability

of extinction, not just one. The Permian extinction reset the clock of community evolution, and it resumed a little faster than before. Similarly, control by the physical environment does not explain the bipartite distribution.

A decrease in probability of extinction can also be explained by an increase in the ratio of positive to negative interactions among taxa. Most plausibly this would involve a decreasing overlap of the adaptive zones⁶ of taxa through time, a pattern which has independent supporting evidence¹. The steeper post-Palaeozoic slope supports this hypothesis because many families did survive into the Triassic and presumably retained the broader aspects of Palaeozoic community structure. Readjustment should then be more rapid than from the initial establishment of animal communities in the Vendian and Cambrian.

The constancy of overall community fitness which is central to the Red Queen hypothesis^{3,7} predicts an approximate constancy in evolutionary rate^{3,8}. That is seen here at the community level, because the ratio of probabilities of extinction among successive equal intervals fluctuates about a constant value. This

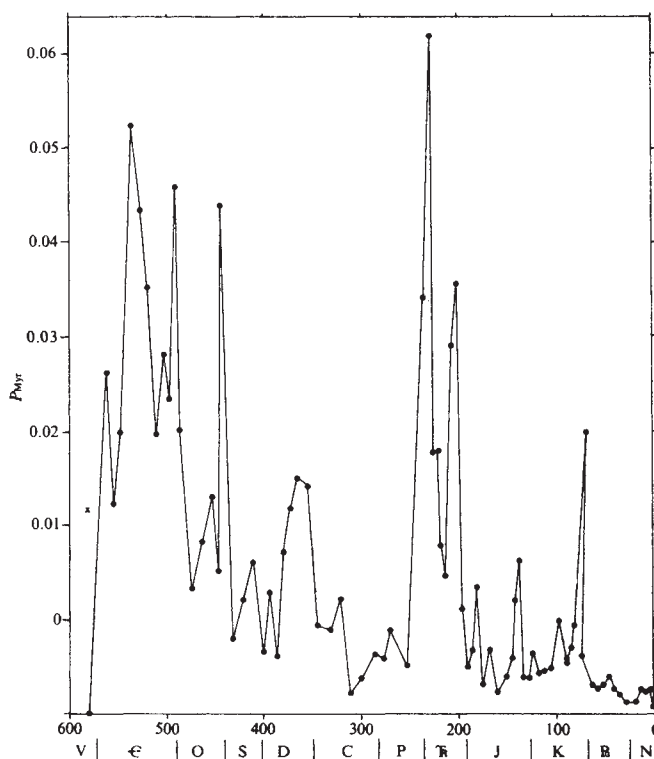


Fig. 2 Probabilities of extinction per Myr (P_{Myr}), as in Fig. 1, on an arithmetic scale. $\times$ is an artificially high value including soft-bodied forms. Abbreviations as in Table 1.

value is determined by the slopes of the regressions in Fig. 1; as it would differ numerically for intervals of different lengths, the value of an average ratio itself has little interest. However, in this way the exponential nature of the decline in probability can be explained in a natural way.

As a subsidiary point, all three radiations (angiosperms⁹, placental mammals¹⁰ and marine animals) for which data are available show unusually low probabilities of extinction, ostensibly zero in each case at the resolution possible, at the very beginning of the major radiation. Both predation¹⁰ and competition were probably unusually low in each of these cases, although density-dependent regulation of population (or metapopulation) density more or less requires that some competition occur among taxa expanding into new adaptive zones¹¹. Thus, initially it is perhaps easier to avoid competition by evolving a new adaptation than it is to stand and fight, evolving adaptations for competition. This should be testable experimentally for suitable organisms in a suitably complex environment.

A cladistic classification would preclude any analysis like that of the present paper.

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1. Simpson, G. G. *The Major Features of Evolution* (Columbia University Press, 1953).
2. Sepkoski, J. J. Jr *Milwaukee Publ. Mus., Contr. Biol. Geol.* **51**, 1–125 (1982).
3. Van Valen, L. M. *Evol. Theor.* **1**, 1–30 (1973).
4. Raup, D. M. & Sepkoski, J. J. Jr *Science* **215**, 1501–1503 (1982).
5. Darwin, C. *On the Origin of Species* (Murray, London, 1859).
6. Van Valen, L. M. *Evolution* **25**, 420–428 (1971).
7. Van Valen, L. M. *Evol. Theor.* **4**, 289–300 (1980).
8. Van Valen, L. M. *J. molec. Evol.* **3**, 89–101 (1974).
9. Doyle, J. A. in *Patterns of Evolution* (ed. Hallam, A.) 501–546 (Elsevier, Amsterdam, 1977).
10. Van Valen, L. M. *Evol. Theor.* **4**, 45–80 (1978).
11. Van Valen, L. M. in *Conceptual Issues in Ecology* (ed. Saarinen, E.) 323–343 (Reidel, Dordrecht, 1982).
12. Alvarez, L. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 627–642 (1983).

Air pollution increases *Aphis fabae* pest potential

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Many observations have been made of increased population densities of herbivorous insects in areas subject to air pollution^{1–3}. However, in the absence of experimental investigations, the significance of most of these observations is not clear and any underlying causal mechanisms remain obscure. The only published studies designed to investigate experimentally the impact of pollutants on plant–herbivore systems involve fumigations with either hydrogen fluoride (HF)⁴ or sulphur dioxide (SO₂)^{5,6} of *Phaseolus vulgaris* and *Glycine max* plants, infested with the Mexican bean beetle, *Epilachna varivestis*: in general the results showed that HF reduced beetle performance whereas SO₂ stimulated it, but the relative importance of direct and indirect effects of the pollutants was not elucidated. We show here stimulatory effects of SO₂ and NO₂ and ambient London air on the growth of the black bean aphid, *Aphis fabae*, and demonstrate that these have been mediated entirely via the host plant.

Annual surveys of *A. fabae* abundance in south-east England have shown consistently higher levels of infestation on field beans, *Vicia faba*, in an area downwind of London and in the proximity of heavy industry, than predicted on the basis of the number of eggs recorded on the winter hosts (M. J. Way, personal communication). We hypothesized that this might be caused by air pollutants drifting into the area and established an experimental programme to investigate the phenomenon.

Fumigation experiments were conducted in a pair of 0.7 m³ Perspex chambers, situated inside a heated glasshouse. The chambers were ventilated with four changes per minute of charcoal-filtered air, and SO₂ or NO₂ was added from a cylinder to give a controlled constant level of pollutant in one chamber. *A. fabae* was reared in culture in a constant temperature room and alatae were removed to clip cages attached to *V. faba* leaves. Two-day-old offspring were removed from the cages for use in each experiment. Subsequent performance of the nymphs was assessed by calculating either final fresh weight or mean relative growth rate (MRGR) on the basis of increases in fresh weight. For each pollutant, two types of experiment were carried out, designed to separate direct and indirect effects on the aphids, respectively. Effects mediated via the plants were investigated by fumigating three-leaf-stage seedlings with 200–500 µg m⁻³ of SO₂ (mean 400 µg m⁻³) or 400 µg m⁻³ NO₂ for 7 days; the plants were then transferred to a controlled temperature room at 20 °C and one nymph was placed on top of each and its MRGR measured over the succeeding 3 days. The possibility that the pollutants had a direct effect on the insects was examined by carrying out 400 µg m⁻³ fumigations with either pollutant while the insects were feeding on artificial diet sachets. At the end of the 3-day fumigation the mean fresh weight per aphid on each sachet was measured and compared between treatment and control.

Table 1 shows MRGR of the aphids grown on post-fumigation plants. Both SO₂ and NO₂ caused significant increases in aphid performance, with MRGR being stimulated by 6.5% and 8.4%, respectively. In contrast, direct fumigation of aphids feeding on the artificial diet produced no significant differences in growth (SO₂ treatment, 126 ± 4 µg; control, 118 ± 6 µg; NO₂ treatment, 90 ± 7 µg; control, 93 ± 5 µg). The MRGR of the diet-fed aphids was not measured directly, because of the problems of disturbance inherent in making repeated measurements on the same animals. However, by taking a mean value for the initial

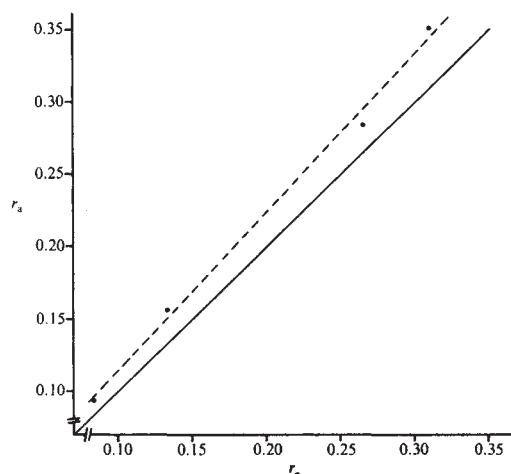


Fig. 1 Relationship between rates of biomass increase for *Aphis fabae* on *Vicia faba*, grown in either ambient London (r_a) or charcoal-filtered (r_c) air. Dashed line, $r_a = 1.086r_c + 0.006$; solid line, $r_a = r_c$.

weight of 2-day-old aphids, the MRGR of diet-fed animals was estimated and shown to be almost identical between the fumigation and control treatments, for both pollutants (Table 1). Although growth was slower overall on the artificial diet, as found by other workers⁷, we consider that the difference is sufficiently small to provide a valid comparison between indirect and direct effects of the pollutants on the aphids.

The fumigations described here used SO_2 and NO_2 concentrations which are higher than the mean annual levels recorded currently in British cities. Therefore, to determine the effect of ambient urban air pollution on the performance of *A. fabae*, another pair of 0.7 m^3 chambers was set up on a roof at Imperial College in South Kensington, central London. These were ventilated with ambient air or the same air which had been passed through a charcoal filter, which removed at least 70% of SO_2 and 50% of NO_2 but no nitric oxide (NO) (S. M. Usher, personal communication). Pots containing three three-leaf-stage *V. faba* plants were infested with *A. fabae* by enclosing each pot in a gauze cage with nine adult alatae. The caged plants were then placed in the chambers, where they were grown for 9–12 days in four separate experiments between May and July 1983. The daily SO_2 concentrations measured at the nearest National Air Pollution survey site (500 m distant) ranged between 34 and $145 \mu\text{g m}^{-3}$ during the experimental periods (S. T. Mossman, personal communication). No local measurements of nitrogen oxides are available, but on the basis of data from elsewhere in central London, the ratio of $\text{SO}_2:\text{NO}_2$ has been estimated⁸ to be about 1:0.7, which would give concentrations of NO_2 at South Kensington of 20–100 $\mu\text{g m}^{-3}$. At the end of each experiment, the aphid offspring in each chamber were counted and weighed and the rate of biomass increase (r) was calculated ($B_t = B_0 e^{rt}$, where B_t = final biomass, B_0 = initial biomass and t = duration of the experiment in days). Different climatic conditions resulted in considerable variation in population growth between experiments, precluding direct comparisons of biomass. Figure 1 shows the relationship between the rates of biomass increase for the ambient air (r_a) and charcoal-filtered air (r_c) treatments. The regression line is significantly greater than a slope of 1 ($P < 0.01$), and there was a 12% increase in r_a over r_c throughout the series of experiments.

The results presented here provide strong evidence that two of the principal phytotoxic air pollutants occurring in the UK can enhance the performance of a serious agricultural pest. Such seemingly small increases in productivity of the insect can, because of the logarithmic nature of population growth, result in large increases in pest populations over small time periods. They will also tend to lead to more frequent escape from control by natural enemies⁹. The fumigation experiments demonstrate that these effects on aphid performance are indirect and medi-

Table 1 Mean relative growth rate over 3 days of *Aphis fabae* subjected to SO_2 or NO_2 , either directly on artificial diet or indirectly via prefumigated plants

		Pollutant	Control	Significance
SO_2	Plant*	0.540	0.507	$P < 0.01$
	Artificial diet†	0.395	0.394	NS
NO_2	Plant‡	0.575	0.530	$P < 0.01$
	Artificial diet§	0.369	0.370	NS

Mean relative growth rate ($\mu\text{g } \mu\text{g}^{-1} \text{ d}^{-1}$) = $(\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{no. of days growth}$. NS, not significant.

* Mean of 5 experiments with 15 replicates per treatment.

† Mean of 2 experiments with 15 replicate sachets per treatment, each with a mean of 10 aphids.

‡ Mean of 4 experiments with 15 replicates per treatment.

§ One experiment with 15 replicate sachets per treatment, each with a mean of 10 aphids.

ated via changes in the plant. We have carried out a preliminary investigation on the nature of these changes by analysing the amino acid spectrum of *V. faba* plants subjected to the 7-day SO_2 fumigation treatments used in the present work. Consistent changes, both negative and positive, were detected for several key amino acids. It is well established that the amino acid composition of a plant has the potential to influence insect performance^{10,11}. However, the significance of the observed changes for *A. fabae* growth is uncertain and will be the subject of future research.

We consider that the results described here indicate a strong possibility that air pollution has a serious economic impact on crop productivity, as a result of increased pest incidence, as well as exerting the more generally recognized direct phytotoxic effects. It is necessary for such work to be extended to a much wider range of pest types and host species to assess the overall importance of air pollution/insect/crop interactions in the field.

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1. Wiackowski, S. K. *Folia for. pol.* **A23**, 175–187 (1978).
2. Port, G. R. & Thompson, J. R. *J. appl. Ecol.* **17**, 649–656 (1980).
3. Villemont, C. *Envir. Pollut.* **A24**, 245–262 (1981).
4. Weinstein, L. H. *J. occup. Med.* **19**, 49–78 (1977).
5. Hughes, P. R., Potter, J. E. & Weinstein, L. H. *Envir. Ent.* **10**, 741–744 (1981).
6. Hughes, P. R., Potter, J. E. & Weinstein, L. H. *Envir. Ent.* **11**, 173–176 (1982).
7. Van Emden, H. F. *Aphid Technology* (Academic, London, 1972).
8. Lane, P. I. thesis, Univ. London (1983).
9. Lawton, J. H. & McNeill, S. in *Population Dynamics* (eds Anderson, R. M., Turner, B. D. & Taylor, L. R.) 223–224 (Blackwell, Oxford, 1979).
10. McNeill, S. & Southwood, T. R. E. in *Biochemical Aspects of Plant and Animal Coevolution* (ed. Harborne, J. B.) 77–98 (Academic, London, 1978).
11. Mattson, W. J. *A. Rev. Ecol. Syst.* **11**, 119–161 (1980).

Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium

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Mycorrhizal infection of plants in many natural vegetation systems arises when uninfected roots make contact with mycelia spreading from infected roots^{1,2}. The result of the infection is that connections are present between the roots of individual plants. It has been shown by autoradiography that these interconnections provide a direct pathway for the transfer of carbon between ecto-mycorrhizal plants^{2,3}. Inter-plant transfer of carbon⁴ and phosphorus^{5–7} has been reported in plants with vesicular-arbuscular (VA) mycorrhizas, but in the absence of

comparable evidence it remains a possibility that in these cases the pathway is indirect, uptake by the fungus of the 'receiver' plant occurring only after leakage of the nutrients from roots of the 'donor'. We now show using autoradiography that transfer of carbon between plants connected by VA mycorrhizal mycelium occurs primarily by the direct hyphal pathway. It is further shown that the magnitude of the transfer is strongly influenced by shading of 'receiver' plants indicating that movement is governed by source-sink relationships.

Seedlings of *Plantago lanceolata* were used as 'donors' both of infection and of labelled carbon. They were first grown in either the non-mycorrhizal (NM) or mycorrhizal (M) condition in trays of dune sand partially sterilized (PS) by irradiation. Two categories of M plants were produced, one by inoculation with field collected root pieces (unspecific inoculum) and the other with surface-sterilized spores of *Glomus caledonium* (specific inoculum). After 5 weeks both the M and NM donor plants were transferred to fresh PS sand in transparent plastic Petri dishes, to which also were added three aseptically grown seedlings of *Festuca ovina*. Twelve dishes of each category of donor (NM, M field inoculum, M specific inoculum) were established. Inspection of *Festuca* roots in randomly selected dishes after 6 weeks revealed infection levels of 30–50% in M non-specific and 15–20% in M specific inoculum categories. The sand surface was then coated with warm molten paraffin wax, to ensure a seal around the bases of all plants and around the dish circumference. Immediately before application of isotope to donor plants, the receiver plants in six dishes of the NM and six of the field inoculum categories were subjected to shade treatments. Darkness was obtained by loosely covering *Festuca* shoots with aluminium foil, half-shade by covering the shoots with muslin cloth, the remaining dishes being exposed to full illumination. Shoots of donor plants were then sealed in transparent plastic boxes into each of which 30 μCi of $^{14}\text{CO}_2$ was released. The plants were exposed to the isotope for 2 days after which time the shoots were excised, and the root systems in three dishes of each of the three categories of plants were prepared for autoradiography by gently removing sand under a fine jet of water. Roots, and in the case of M plants, their mycelial interconnections were then photographed through the transparent dish base before autoradiographic stripping film was applied to the roots which were exposed to the film for 2 days. The remaining M field inoculum and NM plants were harvested, ground in liquid nitrogen and digested in NCS tissue solubilizer before ^{14}C levels of their roots and shoots were determined quantitatively by liquid scintillation counting.

Roots of *Plantago* and *Festuca* can be readily distinguished on the basis of size (Figs 1a, 2a) and in the inoculated dishes a network of hyphae is seen to form frequent interspecific linkages between plants (Figs 2, 3). Autoradiographs reveal that whereas the roots of NM donor plants are strongly labelled no activity is detected in those of *Festuca* even where they are in

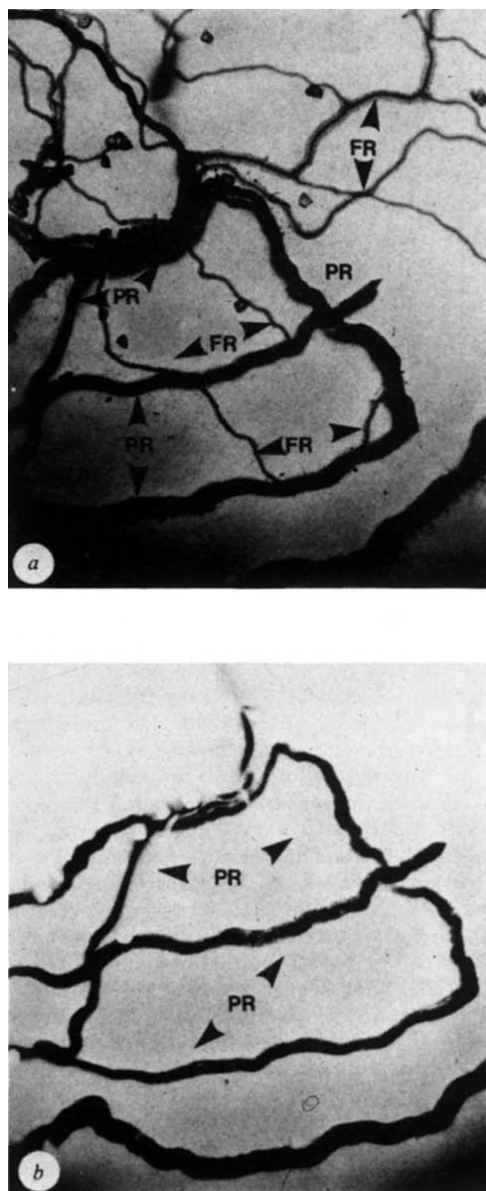


Fig. 1 a, Light micrograph of roots of an NM system after removal of sand showing closely intertwined roots of *Plantago* (PR) which are relatively robust, and *Festuca* (FR) ($\times 15$). b, Autoradiograph of same area as a showing accumulation of radioactivity in the donor, *Plantago* (PR), and complete absence of detectable transfer even into the most closely associated *Festuca* roots ($\times 15$).

Table 1 Radioactivity in shoots and roots of receiver plants (*Festuca ovina*) under three light regimes

Treatment	Receiver category					
	<i>Festuca</i> mycorrhizal			<i>Festuca</i> non-mycorrhizal		
	Activity in root (d.p.m. per mg dry wt)	Activity in shoot (d.p.m. per mg dry wt)	Activity in whole receiver plant as % of that in donor	Activity in root (d.p.m. per mg dry wt)	Activity in shoots (d.p.m. per mg dry wt)	Activity in whole receiver plant as % of that in donor
Full light	8,908* $\pm$ 3,530	363† $\pm$ 242	0.015	656† $\pm$ 403	147† $\pm$ 194	0.0019
Half Light	18,072* $\pm$ 7,647	479† $\pm$ 75	0.048	264† $\pm$ 365	229† $\pm$ 372	0.0010
Dark	57,218* $\pm$ 12,372	51† $\pm$ 38	0.112	171† $\pm$ 100	ND†	0.0005

Counts are expressed as d.p.m. mg dry weight (with 95% confidence limits) and as percentage of the total activity in the donor plants at harvest ($n = 6$). ND, no counts above background detected.

* Significant difference between this and all other values of activity at both treatment and category levels at $P < 0.05$.

† Significant difference between this and other value so marked at $P < 0.01$.

‡ Value not significantly different from other treatments in the same category.

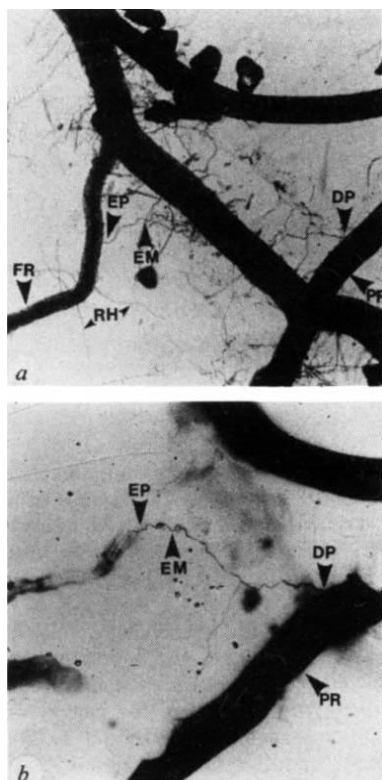


Fig. 2 *a*, Light micrograph of roots in a specific inoculum system showing *Plantago* 'donor' root (PR), *Festuca* 'receiver' root (FR) with many root hairs (RH), and interconnecting endophyte mycelium (EM) of *Glomus caledonium* ($\times 40$). *b*, Autoradiograph of same area as *a* showing heavy labelling of donor root (PR) and of endophyte mycelium (EM). Mycelial linkage can be seen from departure point (DP) on donor root to entry point (EP) on receiver root. Radioactivity in receiver roots is restricted to area of developing infection around the fungal entry point (EP) ($\times 40$).

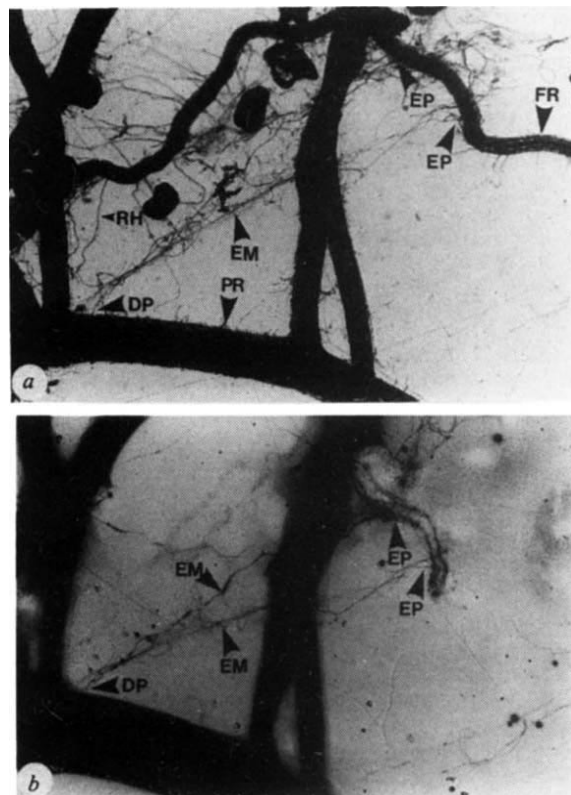


Fig. 3 *a*, Light micrograph of roots of a field inoculum system with typical fascicle of hyphae (EM) passing from departure point (DP) on 'donor' root (PR) and branching to form an entry point (EP) in the upper *Festuca* root (FR) ($\times 40$). *b*, Autoradiograph of same area as *a* showing accumulation of radioactivity in the hyphal fascicle (EM), at the entry point (EP) and in the infected part of the *Festuca* root ($\times 40$).

close physical contact with *Plantago* (Fig. 1*b*). Indeed, there is little evidence of loss of carbon through leakage from roots of either NM or M donor plants. In the M systems, however, label has moved from donor roots into the external mycelium and to connected receiver roots where it has accumulated around newly formed infections of the *G. caledonium* systems (Fig. 2*b*) and in the vesicles and arbuscules of the more advanced field inoculum infections (Fig. 4*b*). Labelling of mycelium is most pronounced in the coarse hyphae of *G. caledonium* (Fig. 2*b*). The finer hyphae of the field inoculum systems are often grouped together in fascicles running between roots. Chlamydospores developing on these hyphae become sinks for accumulation of label (Fig. 4*a, b*) confirming an earlier report⁸, which was based upon counting methods. Again, only infected roots accumulate label (Fig. 4*b*) most of which can be shown by subsequent staining to be restricted to vesicles and arbuscules. This pattern of tracer movement indicates that in VA, as in ectomycorrhizas², transfer of carbon between plants occurs primarily by a direct hyphal pathway. Slow leakage may yield some assimilate to scavenging mycorrhizal hyphae and so provide an additional indirect or facilitated uptake by receiver plants but the significance of such a pathway is likely to be relatively small not only because leakage is restricted but because of intense competition for such materials amongst the microorganisms of the rhizosphere.

Quantitative analysis of radioactivity in the plants (Table 1) confirms the occurrence of assimilate transfer between mycorrhizal seedlings. While label is scarcely detectable in NM plants, levels of activity in M receiver roots of both categories is high and in darkened M plants is six times greater than in

those which are fully illuminated. Since the quantity of label moved is strongly influenced by shading it is likely that levels of transfer are largely governed by source-sink relationships. Relatively little activity is detected in shoots of either M or NM receiver plants, probably not only because of the evident capacity of the endophyte mycelium in the root to accumulate labelled material but also because the period of exposure to the isotope was short. Nevertheless the fact that levels of carbon in whole receiver plants can reach over 0.1% of those in donors, even in this limited time span, indicates that whole plants may be to some extent interdependent in terms of assimilate supply. The detection of isotope in the receiver does not, by itself, prove net movement of carbon to these plants, and longer term studies, now being carried out, are required to quantify further the assimilate transfer patterns.

Since seedlings frequently spend their early lives in adverse light environments these observations are likely to be of great ecological and physiological significance. It has been suggested previously⁹⁻¹¹ that the mycorrhizal fungus may form a vigorous sink for host assimilate in the individual infected plant, but in nature plants occur in communities in which individuals are interconnected at both intra- and interspecific levels, through their mycorrhizas. Our results indicate that carbon will move between these plants along gradients caused by differences of illumination and hence of sink strength. Early mycorrhizal infection would provide enhanced nutrient supply but could be critically disadvantageous in terms of the carbon drain imposed by the fungus. This study shows that seedling infection may be sustained by direct transfer of assimilates from neighbouring mature plants which are more likely to be fully illuminated and

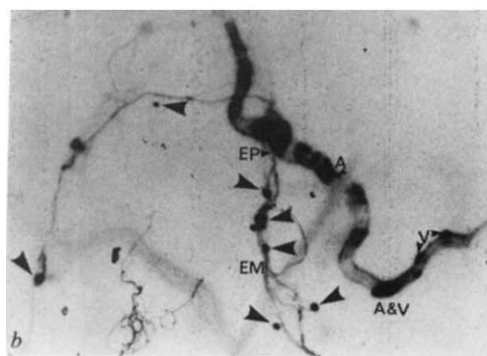
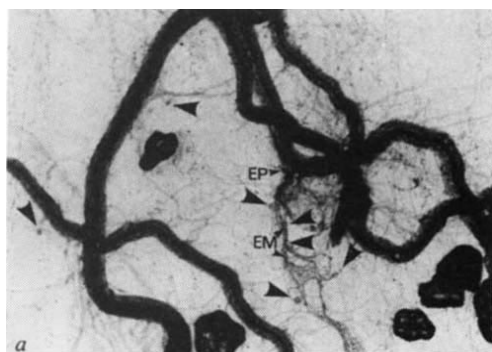


Fig. 4 *a*, Group of *Festuca* roots from field inoculum system showing infection point (EP) arising from a fascicle of hyphae (EM) in which numerous chlamydospores (arrowed) are developing ($\times 40$). *b*, Autoradiograph of this group showing accumulation of radioactivity at the entry point (EP), in vesicles (V) and arbuscules (A) along the infected root, in the mycelial fascicle (EM) and in the developing spores. Uninfected roots contain little or no labelled material ($\times 40$).

from which infection has spread. There is the further possibility that during the critical establishment phase the entire seedling may live for a period in partial heterotrophic dependence upon its neighbours. The extent of this dependence will be revealed in experiments now being conducted over a longer time period.

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1. Read, D. J., Koucheki, H. K. & Hodgson, J. H. *New Phytol.* **77**, 641–651 (1976).
2. Brownlee, C., Duddridge, J. A., Malibari, A. & Read, D. J. *Pl. Soil* **71**, 433–443 (1983).
3. Reid, C. P. P. & Woods, F. W. *Ecology* **50**, 178–187 (1969).
4. Hirrel, M. C. & Gerdemann, J. W. *New Phytol.* **83**, 731–738 (1979).
5. Heap, A. J. & Newman, E. I. *New Phytol.* **5**, 173–180 (1980).
6. Whittingham, J. & Read, D. J. *New Phytol.* **90**, 277–284 (1982).
7. Chiariello, N., Hickman, J. C. & Mooney, H. A. *Science* **217**, 941–943 (1982).
8. Ho, I. & Trappe, J. M. *Nature* **244**, 30–31 (1973).
9. Cox, G., Sanders, F. E., Tinker, P. B. & Wild, J. A. in *Endomycorrhizas* (eds Sanders, F. E., Mosse, B. & Tinker, P. B.) 297–312 (Academic, London, 1975).
10. Buwalda, J. G. & Goh, K. M. *Soil. Biol. Biochem.* **14**, 103–106 (1982).
11. Snellgrove, R. C., Splittstoesser, W. E., Stribley, D. P. & Tinker, P. B. *New Phytol.* **92**, 75–87 (1982).

X-ray diffraction evidence that actin is a 100 Å filament

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The shape of the actin monomer has been determined by X-ray crystallography¹ and image analysis² to be a prolate ellipsoid consisting of two domains. A key structural question is how this monomer is assembled into the biologically important actin filament. Two completely different classes of models of F-actin have recently appeared. In one, the long axis of the monomer is oriented parallel to the filament axis^{1,2}, while in the other it is nearly perpendicular to that axis^{3–5}. Fowler and Aebi⁶ have argued that a proper assessment of the diameter of the actin filament can distinguish between the correctness of these models, since the 'perpendicular' model is about 95 Å in diameter, while the 'parallel' model is about 75 Å in diameter. Using electron microscopy, they have determined the diameter to be between 70 and 80 Å, consistent with their model. Egelman and DeRosier³ also provided evidence from electron microscopy that the filament was about 95 Å, consistent with their perpendicular model. However, all specimens prepared for electron microscopy are susceptible to various artefacts induced by the preparation procedure. X-ray diffraction of specimens in solution allows one to examine objects in their native state. Data from X-ray diffraction of actin filaments in live muscle are presented here which show that a reasonable value for the diameter of F-actin is between 95 and 100 Å.

The strongest feature in the muscle X-ray pattern arising from the thin filament is the 59 Å layer line (the measured amplitude distribution is shown in Fig. 1A). Because of the different helical symmetries of actin and myosin in muscle, the 59 Å layer line arises only from the thin filament and is related to the distribution of density along the 59 Å left-handed one-start helix of the actin filament. Since the long-pitch strands of tropomyosin are nearly perpendicular to this short-pitch actin helix, tropomyosin will make almost no contribution at low resolution to the diffracted intensity along this layer line⁷. Therefore, by using the phases determined by electron microscopy of negatively stained filaments³ one can use the X-ray amplitudes to generate a projection of the actin density along this 59 Å helix. Computed transforms of negatively stained filaments showed that the phase was relatively flat across the single broad amplitude peak (considerations of phase continuity show that this must be the case for an object the approximate diameter of F-actin). The resulting helical projection is presented in a one dimensional form in Fig. 1B. The X-ray data used were of limited resolution (extending out to 0.020 Å⁻¹ from the meridian), but it is clear that any higher resolution data (out to 0.030 Å⁻¹) are weak and therefore could not dramatically change the distribution. A comparable helical projection (using the same resolution cutoff) obtained from the model of Egelman and DeRosier is also shown.

The model data match the X-ray data well, the only difference being that the observed data extend out further in radius than the model. The X-ray data shown are consistent with a filament having significant mass extending out to a 100 Å diameter, and are completely inconsistent with a 70–80 Å diameter filament. A comparable plot to Fig. 1B (data not shown) has been made using the recently published layer line intensities from a relaxed smooth muscle⁸. The intensity distribution shown by Tajima *et*

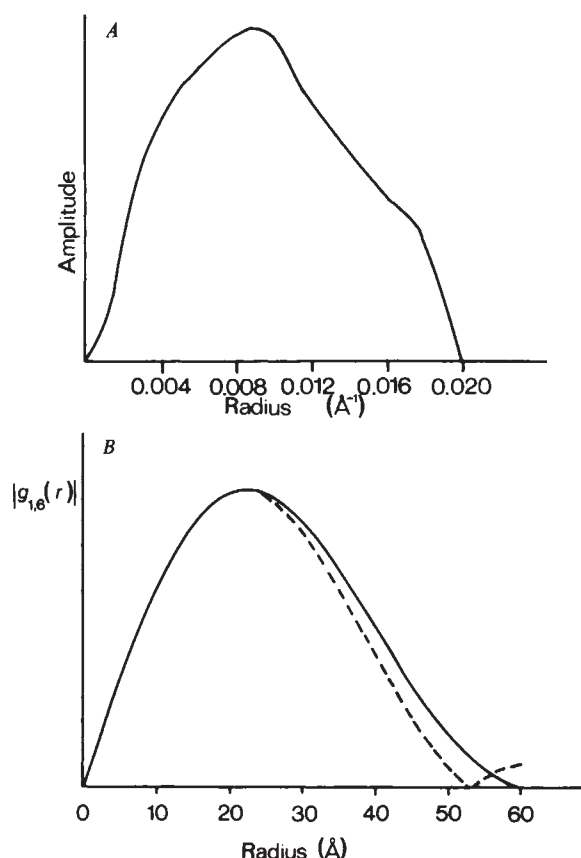


Fig. 1 A, The integrated intensity of the 59 Å layer line from the X-ray diffraction pattern of live relaxed frog sartorius muscle was measured, and a background was subtracted. The resulting amplitude distribution is shown. B, The function $g_{1,6}(r)$ (ref. 15) has then been computed using Fourier-Bessel inversion of the data shown in (A) (solid curve). A corresponding plot from the model of Egelman and DeRosier³ is also shown (dashed curve). This function is proportional to the modulation of F-actin density when the filament structure is projected along the 59 Å left-handed helix. Because the true radial density distribution of the filament (derived from the equator) has not been added in, this function is zero at the filament axis. The density distribution for the model extends beyond the 49 Å outer radius of the model because of the resolution cutoff which has been imposed to make the two plots comparable.

*al.*⁸ extends out to 0.024 Å^{-1} on the 59 Å layer line, and the resulting helical projection agrees even better with the model. This suggests that the cutoff of our observed intensity at 0.020 Å^{-1} is responsible for the difference which exists between the model helical projection and the X-ray derived projection.

A second line of X-ray evidence concerns the radius of gyration of the actin filament. This quantity is equal to the square root of the second moment of the average cross-sectional density distribution:

$$\left(\frac{\int r^2 \rho(r, \theta) r dr d\theta}{\int \rho(r, \theta) r dr d\theta} \right)^{1/2}$$

It has been measured to be $25 \pm 2 \text{ Å}$ (ref. 9 and R. Mendelson, personal communication). A homogeneous cylinder (whose mean radial density distribution is given by a box function) will have a radius of gyration equal to $r_{\text{max}}/\sqrt{2}$, where r_{max} is the outer radius of the cylinder. Thus, the 25 Å value obtained implies that the radius of gyration of actin is the same as that of a homogeneous cylinder of maximum radius $\sqrt{2} \times 25 \text{ Å}$, or 71 Å in diameter. This value was cited by Fowler and Aebi⁶ as being consistent with their 70–80 Å model. But we know, in fact, that actin is far from a solid cylinder. For one thing, it has a deep helical groove along the 59 Å helix which gives rise to the strong and broad peak along the 59 Å actin layer line. This

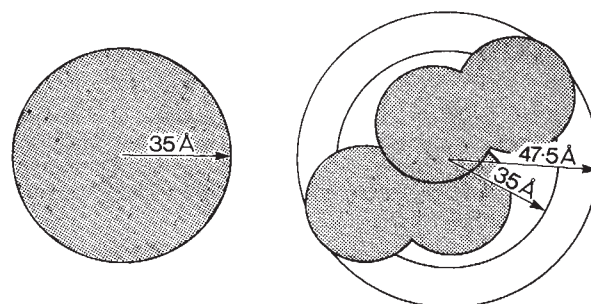


Fig. 2 The radial density distribution of two different models having the same radius of gyration (25 Å) as that experimentally determined for actin⁹ are compared. A, A homogeneous solid cylinder. B, Two actin subunits from the model of Egelman and DeRosier³, looking down the filament axis. The model uses two symmetrical spheres in the absence of any better information on which domain is the larger. The model is basically enclosed by a cylinder of 95 Å in diameter. The model's radius of gyration (25.4 Å) is equal to that of the solid cylinder shown in A.

is the most characteristic feature of both electron microscope transforms and X-ray patterns of actin. A consideration of how the actin subunit can be packed into a filament having such a groove suggests that proportionately more mass has been removed from the 'ideal' homogeneous cylinder at higher radius than at lower radius. This means that the mean radial density distribution will fall with radius. Therefore, the fact that actin has a radius of gyration equivalent to that of a homogeneous cylinder of 71 Å diameter is a strong argument that the maximum diameter of the actin filament is significantly greater than 71 Å. Figure 2 compares the radial density distribution of the 'perpendicular' actin model with that of a homogeneous cylinder having the same radius of gyration. The model of Egelman and DeRosier shown has a radius of gyration of 25.4 Å, in complete agreement with the measured value.

The third avenue of X-ray evidence arises from the early observations^{10,11} of the existence of a sixth layer line meridional reflection in the X-ray diffraction pattern of pure F-actin gels. Such a reflection is 'forbidden' by helical symmetry, and must arise from a perturbation of the axial distribution of mass in the actin filament. Lednev interpreted this feature in terms of subunits within the actin filament being in different states. Egelman and DeRosier¹² extended this, using evidence from Mg^{2+} paracrystals and the bending of stereocilia¹³ to show that the subunit within the actin filament possessed a variable tilt. They also argued that this feature, mediated by cross-bridging proteins in the stereocilia, arose only from inter-filament packing interactions in Mg^{2+} paracrystals. It is therefore important that Lednev first observed the existence of this reflection when the mean inter-filament spacing in a pure F-actin gel was slightly over 100 Å (ref. 10). At larger spacings this reflection disappeared. This was originally interpreted by Lednev to mean that this deformation of the filament structure occurred when filament surfaces were about 40 Å apart, assuming a 70 Å diameter filament (a traditional assumption). This observation is more consistent with a picture of direct contacts between 'fat' actin filaments inducing tilts of subunits within the filament. The original explanation, of filament conformation being mediated by long range forces, seems much less plausible.

Thus our determination of actin filament diameter using data from X-ray diffraction shows that the actin filament must have a diameter of about 100 Å, and that the perpendicular arrangement of subunits in the filament is required to account for this large outer diameter. Further, this orientation of the monomer is supported by image analysis of isolated actin filaments³, S-1 decorated actin⁴, and single-layered paracrystals of thin filaments⁵. The most likely origin of the parallel model of Smith *et al.*² remains the superposition problem associated with multiple layer paracrystals¹⁴.

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Note added in proof: The radius of gyration of F-actin has recently been remeasured and found to be $25.7 \pm 0.5 \text{ \AA}$ (P. Matsudaira, J. Bordas and M. Koch, personal communication). This is in agreement with the cited value⁹, but with a significantly smaller error.

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1. Suck, D., Kabsch, W. & Mannherz, H. G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4319–4323 (1981).
2. Smith, P. R., Fowler, W. E., Pollard, T. D. & Aebi, U. *J. molec. Biol.* **167**, 641–660 (1983).
3. Egelman, E. H. & DeRosier, D. J. *J. molec. Biol.* **116**, 623–629 (1983).
4. Taylor, K. A. & Amos, L. A. in *Actin: Its Structure and Function in Muscle and non-Muscle Cells* (eds dos Remedios, C. & Barden, J.) 25–26 (Academic, Sydney, 1983).
5. O'Brien, E. J., Couch, J., Johnson, G. R. P. & Morris, E. P. in *Actin: Its Structure and Function in Muscle and non-Muscle Cells* (eds dos Remedios, C. & Barden, J.) (Academic, Sydney, 1983).
6. Fowler, W. E. & Aebi, U. *J. Cell Biol.* **97**, 264–269 (1983).
7. Parry, D. A. D. & Squire, J. M. *J. molec. Biol.* **75**, 33–55 (1973).
8. Tajima, Y., Kamiya, K. & Seto, T. *Biophys. J.* **43**, 335–343 (1983).
9. Hartt, J. & Mendelson, R. *Fedn Proc.* **39**, 1728 (1980).
10. Lednev, V. in *Structural Basis and Regulation of Biological Movement* [Russian] (Nauka, Moscow, 1980).
11. Hanson, J., Lednev, V., O'Brien, E. J. & Bennett, P. M., *Cold Spring Harb. Symp. quant. Biol.* **37**, 311–318 (1972).
12. Egelman, E. H. & DeRosier, D. J. in *Actin: Its Structure and Function in Muscle and non-Muscle Cells* (eds dos Remedios, C. & Barden, J.) 17–24 (Academic, Sydney, 1983).
13. Tilney, L. G., Egelman, E. H., DeRosier, D. J. & Sander, J. C. *J. Cell Biol.* **96**, 822–834 (1983).
14. Egelman, E. H., Frances, N. & DeRosier, D. J. *J. molec. Biol.* **116**, 605–622 (1983).
15. Klug, A., Crick, F. H. C. & Wyckoff, H. W. *Acta crystallogr. A* **11**, 199–213 (1958).

Direct observation of motion of single F-actin filaments in the presence of myosin

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Actin is found in almost all kinds of non-muscle cells where it is thought to have an important role in cell motility. A proper understanding of that role will only be possible when reliable *in vitro* systems are available for investigating the interaction of cellular actin and myosin. A start has been made on several systems^{1–4}, most recently by Sheetz and Spudich who demonstrated unidirectional movement of HMM-coated beads along F-actin cables on arrays of chloroplasts exposed by dissection of a *Nitella* cell⁵. As an alternative approach, we report here the direct observation by fluorescence microscopy of the movements of single F-actin filaments interacting with soluble myosin fragments energized by Mg^{2+} -ATP.

Nagashima and Asakura reported that single filaments of F-actin decorated with heavy meromyosin (HMM) or S-1 are directly visible under an optical microscope with dark-field illumination, although the filaments without HMM or S-1 decoration are not visible⁶. Recently, S. Asakura (personal communication) found that single filaments of F-actin, without HMM or S-1 decoration, are made visible under a fluorescence microscope by labelling with fluorescein isothiocyanate. Using the fluorescent dye, phalloidin-rhodamine, we could clearly and continuously observe the movement of single filaments of F-actin or F-actin-tropomyosin (TM)-troponin (TN) complex for more than 5 min without photobleaching. Phalloidin-rhodamine is most convenient for the present purpose, because it is specifically bound to F-actin without any influence on physiological functions of F-actin^{7,8} and its fluorescence intensity is enhanced 4.2-fold by the binding.

When G-actin is polymerized in the presence of phalloidin-rhodamine, many filaments become visible under a fluorescence microscope. Figure 1 shows micrographs of phalloidin-

Table 1 Flexural rigidity (ϵ) of F-actin and F-actin-TM-TN complexes

Sample	Medium	$\lambda (\mu\text{m}^{-1})$	$\epsilon (10^{-17} \text{ dyn cm}^2)$
FA	+Ca	0.032	6.5
FA+HMM	+Ca	0.042	4.9
	+Ca+ATP	0.059*	3.5*
	–Ca	0.028	7.4
FA+TM+TN	+Ca	0.040	5.2
	–Ca	0.025	8.3
	+Ca	0.045	4.6
FA+TM+TN+HMM	–Ca+ATP	0.027	7.7
	+Ca+ATP	0.053*	3.9*
	+Ca	0.033	6.4

The concentration of HMM was 5 mg HMM ml^{-1} . +Ca, rigor buffer +0.2 mM CaCl_2 ; –Ca, rigor buffer +1 mM EGTA; +Ca+ATP, activating buffer; –Ca+ATP, relaxing buffer. The flexural rigidity, ϵ , was determined by measuring the end-to-end distance, R . About 50 values of R were measured for each filament in a 2–10-s period and the mean square end-to-end distance, $\langle R^2 \rangle$, was related to a parameter of flexibility, λ , by the equation $\langle R^2 \rangle = [2\lambda L - 1 + \exp(-2\lambda L)]/2\lambda^2$ (ref. 16). The most probable values of λ were obtained from a least-square fit to the plots of L versus $\sqrt{\langle R^2 \rangle}$ of more than 10 different filaments 5–10 μm in contour length, L . The flexural rigidity, ϵ , was calculated from the value of λ by the equation $\epsilon = kT/2\lambda$ (ref. 17). FA-PM, F-actin cross-linked by *p*-phenylene-*N,N'*-bis(maleimide) by the method of Knight and Offer¹⁴.

* In the activating buffer, the motion is not a thermal one, but the apparent values of λ and ϵ determined by the above procedure are shown for convenience to compare the extent of bending (see text).

rhodamine-labelled F-actin and F-actin-TM-TN complex in various conditions. All fluorescent filaments have the same brightness and the brightness is uniform along the filament. The number of filaments visible in the field of microscope agrees with the number expected from the concentration of F-actin. The visible filaments are not bundles of F-actin but single filaments of F-actin.

The thermal bending motion of the filaments is observable under the microscope. Table 1 gives the flexural rigidity of F-actin and F-actin-TM-TN complex estimated by the statistical analysis of the end-to-end distance of a number of filaments. The values of the flexural rigidity are in good agreement with those of unlabelled F-actin and F-actin-TM-TN measured by different methods^{6,9–11}. This indicates that the binding of phalloidin does not affect the dynamic property of F-actin although it stabilizes F-actin against depolymerization at low ionic strength or high concentration of KI^{12} . The binding of Ca^{2+} decreased the rigidity of the complex and the binding of HMM further decreased the rigidity in the presence of Ca^{2+} .

When both HMM and Mg^{2+} -ATP are added to the F-actin-TM-TN complex in the presence of Ca^{2+} , the bending motion of long filaments becomes faster and larger in amplitude, and the tumbling motion of shorter filaments becomes conspicuous. During such motions, long filaments are often broken into fragments of up to a few micrometres (Fig. 1d). Figure 2 shows sequences of micrographs taken to compare the bending motion of the F-actin-TM-TN filaments in a solution containing HMM and Mg^{2+} -ATP, in the absence (Fig. 2a) and presence (Fig. 2b) of Ca^{2+} . The filaments in Fig. 2a and b have same length, 10 μm . In the absence of Ca^{2+} (Fig. 2a), the bending motion of the first order mode has an apparent period of 3–6 s, and that of the second order mode has a period of about 0.3 s. Previously, by using dynamic light scattering, the relaxation time of the bending motion of F-actin of 2.5 μm in length was determined to be about 10 ms⁹. The above value, 3–6 s, for the filaments of 10 μm length is reasonable because the relaxation time, τ_n , of the bending motion is proportional to the fourth power of the length, as given by

$$\tau_n = \pi^{-4} (n + (1/2))^{-4} \zeta L^4 \epsilon^{-1} \quad (1)$$

where n is the mode number, L the contour length of the filament, ζ the frictional constant for a structural unit and ϵ the flexural rigidity⁹.

In the presence of Ca^{2+} (Fig. 2b), the apparent period of the bending motion of the first mode decreased to 1–3 s. If the bending is due to thermal brownian motion, the flexural rigidity in the presence of Ca^{2+} must be about twice as large as that in its absence, according to equation (1). However, as found in

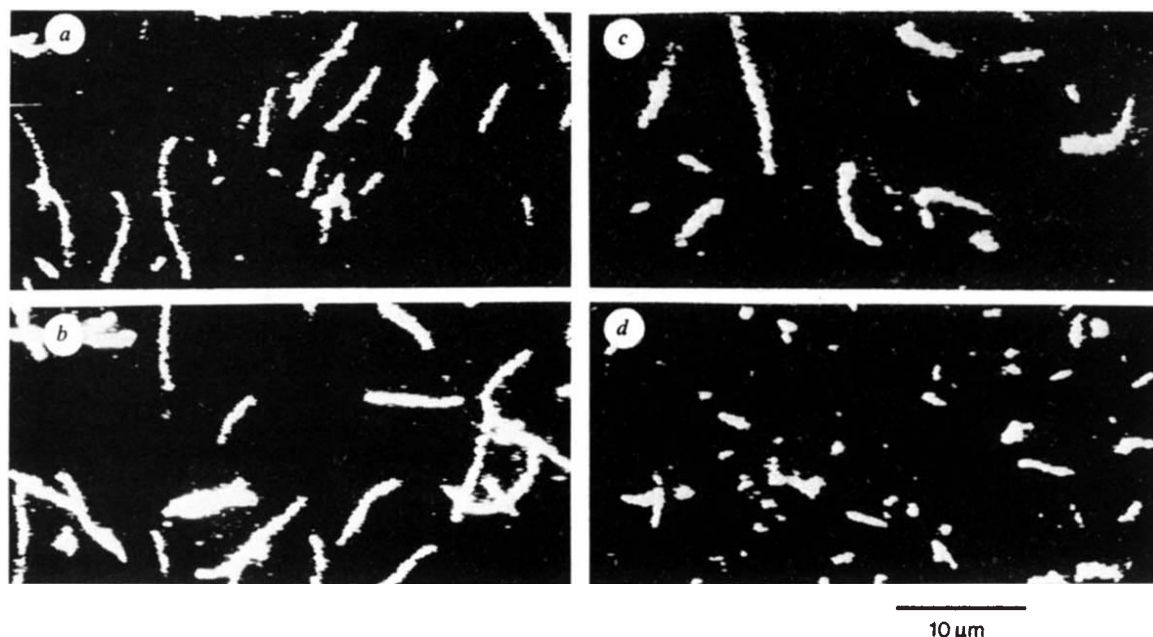


Fig. 1 Micrographs of phalloidin-rhodamine-labelled F-actin: *a* and *b* are for the F-actin in rigor buffer (0.1 M KCl, 5 mM MgCl₂ and 10 mM MOPS pH 7.0) in the absence and the presence of 10 mg HMM ml⁻¹, respectively. *c* and *d* are for the F-actin-TM-TN complexes in relaxing buffer (rigor buffer +1 mM EGTA, 3 mM ATP, 5 mM creatine phosphate and 2 mg creatine kinase per ml) and in activating buffer (rigor buffer +0.2 mM CaCl₂, 3 mM ATP, 5 mM creatine phosphate and 2 mg creatine kinase per ml) containing 10 mg HMM ml⁻¹, respectively. All proteins were obtained from rabbit skeletal muscle and purified according to the method described previously¹⁰. G-actin of 1 µM was polymerized in rigor buffer containing 1 µM phalloidin-rhodamine overnight at room temperature. TM and TN were added to the F-actin solution at a weight ratio of 1:4, respectively. For observation the solutions were diluted 50-fold in buffer and put in a gap between a glass slide and a coverslip of 50 µm in thickness. Final concentration of actin was 20 nM. β-Mercaptoethanol of 0.1–0.5% (v/v) which did not affect actomyosin ATPase activity or muscle contraction was added to the solutions to suppress photobleaching. Fluorescent F-actin or F-actin-TM-TN complexes were observed with a Nikon VFD-TR microscope equipped with epifluorescence optics, a Nikon UV-F ×100 objective (oil immersion, NA 1.3), a 100–200-W mercury arc lamp and a Nikon rhodamine filter set ($\lambda_{EX} \sim 540$ nm, $\lambda_{EM} > 580$ nm). Fluorescent images were recorded on videotape with a high-sensitivity television camera (Ikegami CTC-900) and a video recorder (National Time Laps NV8030).

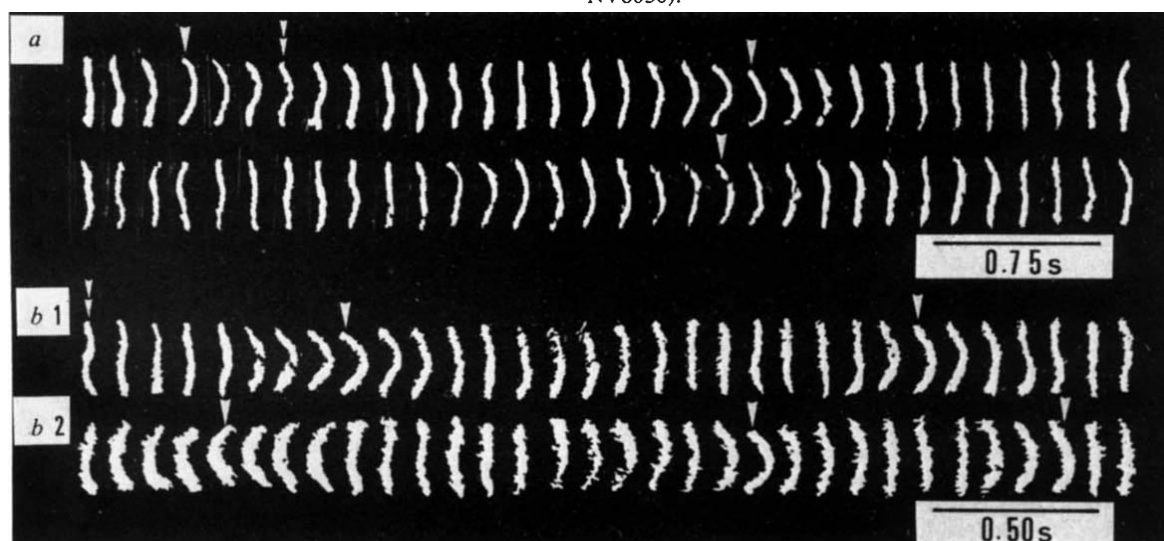


Fig. 2 Sequential micrographs of phalloidin-rhodamine-labelled F-actin having bound TM and TN in relaxing buffer +5 mg HMM ml⁻¹ (*a*) and in activating buffer +5 mg HMM ml⁻¹ (*b*, 1, 2). The intervals between successive images were 0.15 s (*a*) and 0.10 s (*b*, 1, 2), respectively. The contour length of both filaments was 10 µm. Single arrows indicate first-order mode bending motion and double ones second order mode bending motion. As measured by a semiconductor thermometer, the temperature of the solution was 25 °C during illumination. No increase in the temperature of the solution during ATP hydrolysis by HMM, S-1, acto-HMM or acto-S-1 was detectable. The resolution of TV camera (1/60 s) was not high enough to record the rapid motion of filaments, especially in activated state (*b*).

Figure 2*a* and *b*, the average amplitude of bending of the filaments is distinctly larger in the presence of Ca²⁺ than in its absence. Therefore, we conclude that more rigid filaments in the presence of Ca²⁺ were forcibly bent by interaction with HMM and ATP. Conversely, it is also possible to conclude that the bending motion of the filament which is more flexible in the presence of Ca²⁺ was accelerated by interaction with HMM and Mg²⁺-ATP. The latter case is more likely because the F-actin-

TM-TN complex is more flexible in the presence of Ca²⁺ as shown in Table 1. In the present conditions (0.1 M KCl, 20 nM actin and 15 µM HMM), the rate of hydrolysis of ATP is estimated to be about 1 mole per mole actin per s (ref. 13). That is, each actin monomer is attacked by energized HMM (HMM-ADP-P_i) about once per second. The energy liberated by the hydrolysis may be (partially) transferred to F-actin to induce its active motion.

Similar results have been obtained by using pure F-actin instead of the F-actin-TM-TN complex and we have preliminary evidence that S-1 has the same effects as HMM on the motion of F-actin and the F-actin-TM-TN complex. The two-headed structure of HMM is not absolutely necessary to produce faster and larger bending motion of F-actin and the complex.

The effect of cross-linking of actin subunits by *p*-phenylene *N,N'*-bis(maleimide) (PM) on the motion of F-actin was also examined. Previously, by applying this reagent, Knight and Offer showed that the cross-linking did not change the ability of F-actin to activate myosin ATPase and induce superprecipitation, and suggested that relative motion of actin subunits was not required for the activation¹⁴. According to our direct observation, however, the cross-linking by this reagent does not restrict the motion of F-actin and the cross-linked F-actin has the same value of the flexural rigidity as before cross-linking (Table 1).

The present finding suggests that in an activated state, myosin heads induce local conformational changes in F-actin such as distortion or rotation of actin monomers and loosening of actin-actin bonds^{8,15}, which result in fast and large bending in F-actin filaments. Since F-actin has a structural polarity, the motion in F-actin could be asymmetric, if it occurred during interaction with HMM or S-1 in the presence of Mg^{2+} -ATP. However, the interaction between single F-actin filaments and soluble myosin fragments did not produce directional movement. When F-actin

filaments function in living cells, they are usually found as assemblies with same polarity and often supported by some backbone such as membrane. Therefore, higher organizations of F-actin such as polar bundles may be indispensable to transformation of the activated motion in F-actin into an effective sliding force.

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1. Opatka, A. & Tirosh, R. *Biochim. biophys. Acta* **305**, 683-688 (1973).
2. Yano, M., Yamamoto, S. & Shimizu, H. *Nature* **299**, 557-559 (1982).
3. Kuroda, K. & Kamiya, N. *Proc. Japan. Acad.* **51**, 774-777 (1975).
4. Higashi-Fujime, S. *J. Cell Biol.* **87**, 569-578 (1980).
5. Sheetz, M. P. & Spudich, J. A. *Nature* **303**, 31-35 (1983).
6. Nagashima, H. & Asakura, S. *J. molec. Biol.* **136**, 169-182 (1980).
7. Wulf, E., Dehoben, A., Bautz, F. A., Faulstich, H. & Wieland, Th. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4498-4502 (1979).
8. Pröchniewicz-Nakayama, E., Yanagida, T. & Oosawa, F. *J. Cell Biol.* (in the press).
9. Fujime, S. *J. Phys. Soc. Japan* **29**, 751-759 (1970).
10. Yanagida, T. & Oosawa, F. *J. molec. Biol.* **126**, 509-524 (1978).
11. Yoshino, S., Umazume, Y., Natori, R., Fujime, S. & Chiba, S. *Biophys. Chem.* **8**, 317-326 (1978).
12. Wieland, Th. & Faulstich, H. *CRC crit. Rev. Biochem.* **5**, 185-260 (1978).
13. Eisenberg, E. & Moos, C. *J. biol. Chem.* **242**, 2945-2951 (1967).
14. Knight, P. & Offer, G. *Biochemistry* **19**, 4682-4687 (1980).
15. Yanagida, T. in *Cross-bridge Mechanism in Muscle Contraction* (eds Pollack, G. H. & Sugi, H.) (Plenum, New York, in the press).
16. Landau, L. D. & Lifshitz, E. M. *Statistical Physics*, 478-482 (Pergamon, London, 1958).
17. Harris, R. A. & Hearst, J. E. *J. chem. Phys.* **44**, 2595-2609 (1966).

Microtubules with more than 13 protofilaments in the dividing nuclei of ciliates

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Microtubules are largely composed of proteins called tubulins. These are stacked in linear arrays called protofilaments (p). Most microtubules have precisely 13p (ref. 1). The 'incomplete' B and C microtubules (10 or 11p) of cilia, flagella, basal bodies and centrioles are widespread exceptions^{2,3}. Very few examples of 'complete' microtubules with more, or less, than 13p have been found⁴⁻⁷. However, the 'ciliate cell' includes a larger number of highly differentiated types of microtubule arrays than most other cell types⁸⁻¹⁰. The present study was undertaken to ascertain whether there is variation in p number in two ciliates. In both, all complete cytoplasmic microtubules examined have 13p but microtubules with 13-16p are present in the nucleoplasm of dividing nuclei. These features are probably common to ciliates in general because the free-living hymenostome *Paramecium tetraurelia* and the parasitic heterotrich *Nycotrichus ovalis* are not closely related in terms of taxonomic criteria¹¹ or life-style.

During the division of a ciliate, two main categories of microtubule arrays can be distinguished: cytoplasmic and nucleoplasmic. Nuclear envelopes remain intact and large numbers of aligned nucleoplasmic microtubules are associated with the marked elongation of dividing nuclei (Fig. 1)^{12,13}. Protofilaments can usually be detected in cross-sections of microtubules fixed in the presence of tannic acid³. However, ciliates possess a substantial pellicle, and nuclear envelopes provide an additional barrier to tannic acid access so far as nucleoplasmic microtubules are concerned. Because of this we have had to devise a protocol for making the organisms permeable without loss of alignment or disassembly of microtubules prior to fixation (Fig. 2).

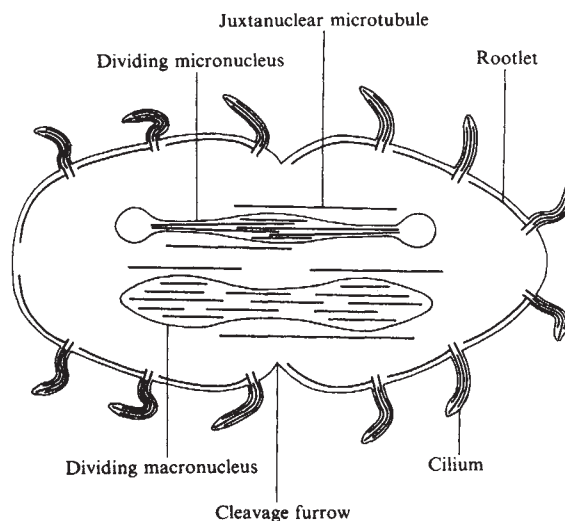


Fig. 1 The general distribution of cytoplasmic and nucleoplasmic microtubules in a dividing ciliate. The cell membrane and nuclear envelopes are depicted by thin lines. The thicker lines show the locations and orientations of microtubules. For clarity, specialized cortical organelle complexes that include microtubules such as mouthparts have been omitted.

Cytoplasmic microtubules with 13p include those that contribute to the cortical cytoskeletal complex such as cytopharyngeal microtubules¹⁴ (Fig. 2a) and the A and central pair microtubules of cilia, as well as the more deeply situated juxtanuclear microtubules (Fig. 1) that run very closely alongside the outer surfaces of dividing nuclei (Fig. 3f). In *Paramecium* there is a set of cortical 'cytoskeleton' microtubules¹⁵ that is probably a type of basal body rootlet array. Like most nucleoplasmic microtubules, and unlike most of the other cytoplasmic microtubules, they are only present during cell division and apparently promote elongation [of the dividing cell body]^{8,15,16}. Nevertheless, they have 13p.

The p number that predominates in dividing micronuclei changes as mitosis proceeds. This is correlated with a marked switch in the organization of nucleoplasmic microtubules during micronuclear mitosis. During the earlier stages (prophase-metaphase) spindle architecture is similar to that of mitotic spindles

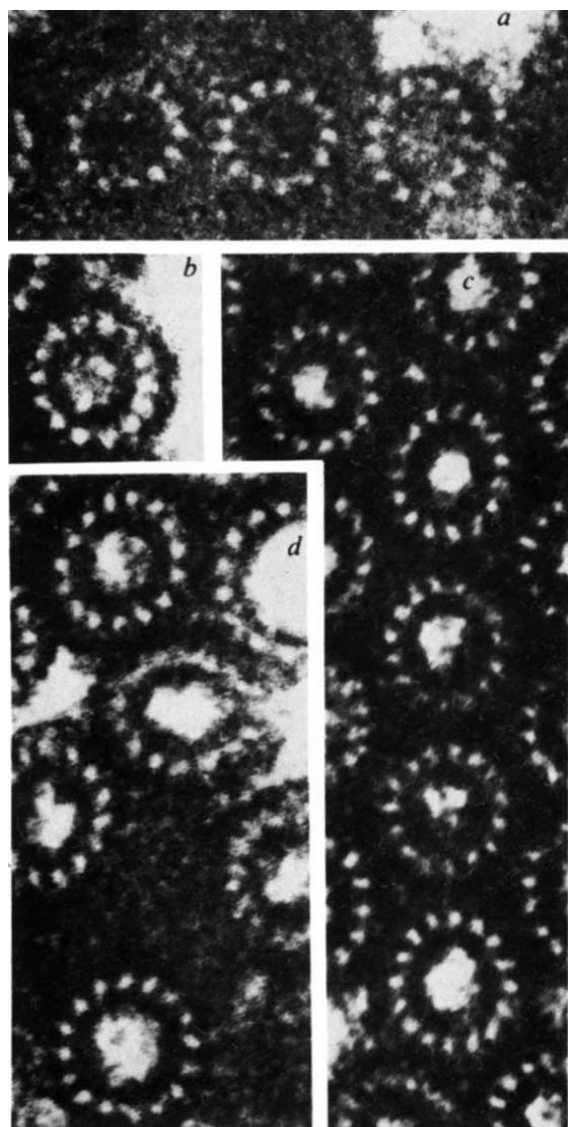


Fig. 2 Cross-section of microtubules in *P. tetraurelia*. All $\times 625,000$. Organisms were permeabilized for 90 s by suspension in a solution of the non-ionic detergent NP40 (0.05%) buffered with the microtubule-stabilizing buffer PM2G²⁶ and then fixed for 1 h in a solution buffered with cacodylate (0.15 M, pH 7.2) containing glutaraldehyde (2.5%) and tannic acid (8%). After rinsing with five changes of cacodylate buffer at 4 °C for 12 h the organisms were post-fixed for 30 min in cacodylate-buffered osmium tetroxide (1%). Subsequent preparation for electron microscopy was as described previously¹⁷. *a*, Cytoplasmic microtubules with 13 protofilaments in part of a cytopharyngeal microtubule ribbon. *b–d*, Spindle microtubules in micronuclei at the same late anaphase stage as Fig. 4*b* showing: *b*, a 14 protofilament microtubule; *c*, microtubules with 15 protofilaments and one with 16 (at bottom); *d*, a microtubule with 15 protofilaments (at top) and one with 16 (at bottom).

in general (Fig. 4*a*), although there are no centrioles at spindle poles¹⁷. The vast majority of microtubules have 'conventional' diameters of 24 nm; in *Nyctotherus* (no data for *Paramecium*) they have 13p (Fig. 3*e*) and can be induced to disassemble by exposure to low temperature (0 °C) and antimetabolic drugs¹⁸. However, anaphase B (chromatid separation due to spindle elongation¹⁹) is especially pronounced; micronuclei become dumb-bell-shaped at this stage of mitosis (Fig. 1). A compact bundle of well-aligned microtubules (Fig. 4*b*) extends along the mid-portion of a micronucleus throughout the period of rapid²⁰ and marked elongation. In *Paramecium* most of these microtubules have diameters, up to 32 nm, that are detectably greater than 24 nm^{12,21}, while in *Nyctotherus* they are cold and drug stable¹⁸ unlike those that predominated earlier. Furthermore,

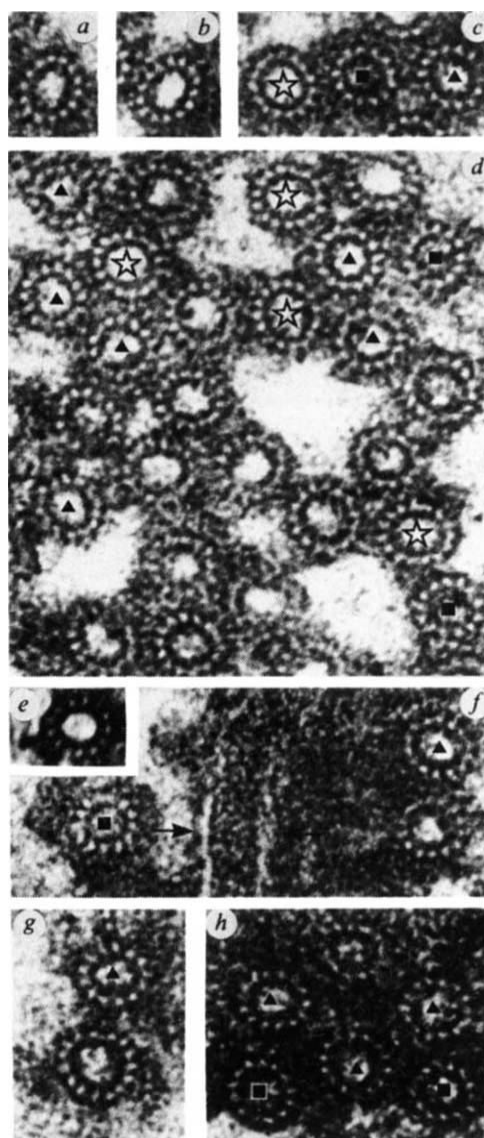


Fig. 3 Cross-sections of microtubules with 13 (■), 14 (▲) and 15 (☆) protofilaments in *N. ovalis* prepared for electron microscopy as described in Fig. 2 apart from the use of NP40 at a concentration of 0.1%. All $\times 370,000$. *a–d*, spindle microtubules in micronuclei at late anaphase showing *a*, *b*, microtubules with 14 protofilaments, *c*, *d*, microtubules with three different protofilament values in close proximity to each other; *e*, a kinetochore microtubule with 13 protofilaments in a metaphase micronucleus; *f*, a juxtanuclear cytoplasmic microtubule (■) positioned closely alongside the envelope (arrows) and nucleoplasmic spindle of an elongating micronucleus, *g*, *h*, microtubules in dividing macronuclei. The microtubule nearest the bottom of *g* has at least 15 protofilaments.

most of the microtubules have more than 13p. Microtubules with 15p predominate in *Paramecium* (Fig. 2*c, d*) and some with 16p (Fig. 2*c, d*), 14p (Fig. 2*b*) and 13p are also present. In *Nyctotherus* most of the microtubules have 14p (Fig. 3*a–d*) but microtubules with 15p and 13p (Fig. 3*c, d*) also occur.

At no stage does macronuclear 'amitosis' involve the construction of a microtubule array that can be regarded as a mitotic spindle, although events are somewhat more orderly than was once supposed²⁰. Microtubules with 13–16p are present (Fig. 3*g, h*) in the dividing macronucleus of *Nyctotherus*. Only 13p microtubules have been detected in *Paramecium* macronuclei to date (however, few unequivocal assessments of p numbers have been possible because the microtubules are not well aligned after organisms have been made permeable and fixed).

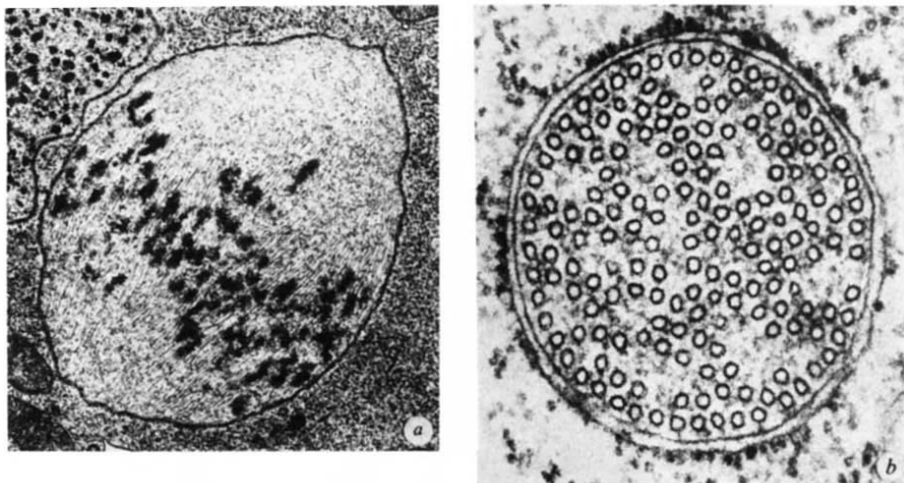


Fig. 4 *a*, Longitudinal section through a micronucleus in *P. tetraurelia* that is either approaching metaphase or has just embarked on anaphase. The clumps of dense material concentrated near the equator represent condensed chromatids. $\times 10,500$. *b*, Cross-section of a nearly fully elongate micronuclear spindle in *P. tetraurelia*. The well-aligned spindle microtubules are surrounded by the nuclear envelope. Differences in microtubule diameters are apparent. $\times 70,000$.

This is the first report of complete microtubules with *p* numbers other than 13 occurring in unicellular organisms and in association with mitosis. It also provides the first example of a situation in which most of the microtubules that assemble in normal conditions *in vivo* are composed of 14*p* (Fig. 3*d*) (only one 14*p* microtubule has previously⁴ been found *in vivo*), and striking instances of variation in *p* number for microtubules in close spatial association (Figs 2*c*, *d* and 3*c*, *d*, *h*). The few examples, and puzzling occurrence, of complete microtubules with more (14*p*–16*p*)^{4,7} or less (11*p*, 12*p*)^{4,6} than 13*p* documented so far (from representatives of four animal phyla including a mammal⁷) suggest no immediately obvious reason why some microtubules have a *p* value other than 13. Variation in *p* number is evidently not a commonly exploited basis for the functional differentiation of microtubules. For example, the complete cytoplasmic microtubules of *Paramecium* and *Nyctotherus* are interconnected in very different ways, are structurally associated with a diversity of other cell components, and almost certainly perform a wide range of different cytoskeletal and mechanochemical functions in the various arrays in which they occur^{8,13,14,20,22}—yet they are all apparently composed of 13*p*.

At least some of the tubulin in the 15*p* spindle microtubules of *Paramecium* is serologically related to some of that in the organism's 13*p* cytoplasmic microtubules¹⁵. However, some of the 'protofilaments' apparent in certain flagellar microtubules after fixation in the presence of tannic acid apparently include proteins other than tubulin^{23,24}. Variation in *p* number may be just a crude preliminary indication of the true diversity of microtubule architecture that abounds.

There is evidence that *p* number is determined *in vivo* when microtubule assembly is initiated and that its specification originates from microtubule-organizing centres²⁵. Is the situation in ciliate nuclei sometimes like that during *in vitro* microtubule assembly when microtubule-organizing centres and *p* number fidelity are both lacking? There is temporal control of the *p* number that predominates during micronuclear mitosis in *Nyctotherus* as reported above. Preliminary indications of positional control for nucleoplasmic microtubules with different *p* numbers in both organisms are emerging. Further analyses of nucleoplasmic microtubules in ciliates should be of especial value in further elucidating the spatial control and functional significance of variation in microtubule protofilament numbers.

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1. Amos, L. A. in *Electron Microscopy of Proteins*, Vol. 3 (ed. Harris, J. R.) 207–250 (Academic, London, 1982).
2. Tilney, L. G. *et al.* *J. Cell Biol.* **59**, 267–275 (1973).
3. Fujiwara, K. & Linck, R. W. *Meth. Cell Biol.* **24A**, 217–233 (1982).
4. Burton, P. R., Hinkley, R. E. & Pierson, G. B. *J. Cell Biol.* **65**, 227–233 (1975).
5. Nagano, T. & Suzuki, F. *J. Cell Biol.* **64**, 242–245 (1975).
6. Chalific, M. & Thomson, J. N. *J. Cell Biol.* **93**, 15–23 (1982).
7. Saito, K. & Hama, K. *J. Electron Microsc. Tokyo* **31**, 278–281 (1982).

8. Jurand, A. & Selman, G. G. *The Anatomy of Paramecium aurelia* (Macmillan, London, 1969).
9. Tucker, J. B. *Nature* **232**, 387–389 (1971).
10. Lynn, D. H. *Biol. Rev.* **56**, 243–292 (1981).
11. Corliss, J. O. *The Ciliated Protozoa: Characterization, Classification and Guide to the Literature*, 2nd edn (Pergamon, New York, 1979).
12. Cohen, J., Beisson, J. & Tucker, J. B. *J. Cell Sci.* **44**, 153–167 (1980).
13. Eichenlaub-Ritter, U. in *Microtubules in Microorganisms* (eds Cappuccinelli, P. & Morris, N. R.) 351–375 (Dekker, New York, 1982).
14. Eichenlaub-Ritter, U. & Ruthmann, A. *Differentiation* **24**, 97–110 (1983).
15. Cohen, J., Adoutte, A., Grandchamp, S., Houdebine, L. M. & Beisson, J. *Biol. Cell* **44**, 35–44 (1982).
16. Sundararaman, V. & Hanson, E. D. *Genet. Res.* **27**, 205–211 (1976).
17. Eichenlaub-Ritter, U. & Ruthmann, A. *Chromosoma* **84**, 701–716 (1982).
18. Eichenlaub-Ritter, U. & Ruthmann, A. *Chromosoma* **85**, 687–706 (1982).
19. Inoué, S. & Ritter, H. in *Molecules and Cell Movement* (eds Inoué, S. & Stephens, R. E.) 3–30 (Raven, New York, 1975).
20. Tucker, J. B., Beisson, J., Roche, D. L. J. & Cohen, J. *J. Cell Sci.* **44**, 135–151 (1980).
21. Tucker, J. B. in *Microtubules* (eds Roberts, K. & Hyams, J. S.) 315–357 (Academic, London, 1979).
22. Plattner, H., Westphal, C. & Tiggemann, R. *J. Cell Biol.* **92**, 368–377 (1982).
23. Linck, R. W. & Langevin, G. L. *J. Cell Sci.* **58**, 1–22 (1982).
24. Linck, R. W. *Ann. N. Y. Acad. Sci.* **383**, 98–121 (1982).
25. Scheele, R. B., Bergen, L. G. & Borisy, G. G. *J. molec. Biol.* **154**, 485–500 (1982).
26. Duerr, A., Pallas, D. & Solomon, F. *Cell* **24**, 203–211 (1981).

Weaver mutation has differential effects on the dopamine-containing innervation of the limbic and nonlimbic striatum

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Ungerstedt¹ observed that the dopamine-containing innervation of the forebrain can be divided into two parts: a nigrostriatal system, originating mainly in the pars compacta of the substantia nigra and innervating the caudoputamen; and a mesolimbic system arising mainly in the ventral tegmental area and innervating the nucleus accumbens and olfactory tubercle. This classification has since been modified and extended with the discovery of the mesocortical dopamine system^{2,3}. The original distinction between nigrostriatal and mesolimbic systems nevertheless was pivotal in suggesting that the basal ganglia are related to limbic as well as to sensorimotor functions, and remains of interest because dopaminergic mechanisms may be implicated not only in the aetiology of sensorimotor impairments such as those of Parkinson's disease⁴, but also in neuropsychiatric disorders such as schizophrenia^{5–7}. The striatal targets of the mesolimbic and nigrostriatal systems are now known to be distinct also in terms of forebrain connections, despite some overlap of fibre projections^{18,19}. The nucleus accumbens–olfactory tubercle region and abutting caudoputamen (together called the 'ventral' or 'limbic' striatum) are characteristically related to limbic parts of the

forebrain, whereas the large remainder of the caudoputamen (the 'dorsal' or 'non-limbic' striatum) is most closely related to sensorimotor regions^{19,20,34}. We report here evidence that the mesolimbic and nigrostriatal systems are differentially affected in the mutant mouse *weaver*, and in particular that dopamine is severely depleted in the dorsal striatum of *weaver* but relatively spared in the ventral striatum. We conclude that dopamine-containing fibre systems innervating the limbic and non-limbic striatum can be influenced separately in genetic disease and that genetic control, whether direct or indirect, may be exerted at the single-gene level.

Weaver (*wv*) an autosomal recessive mutation in the mouse, has been intensively studied because of its effects on the cerebellum. The *weaver* disease is expressed in the cerebellum early in postnatal life when most granule cells die in their proliferative zone after failing to migrate to the internal granular layer⁸. Quite recently it has been discovered that the *weaver* mouse also suffers a severe deficiency of dopamine in the forebrain^{9,10}. This finding prompted the present study, in which we sought to determine the specific extent and location of this neurotransmitter defect in the *weaver* striatum. Biochemical and anatomical observations were made on homozygous *weaver* animals (*wv/wv*) and heterozygous littermate controls (*+wv*) of both sexes obtained by mating C57BL6/CBA (*+wv*) heterozygotes purchased from the Jackson Laboratories (Bar Harbor, Maine). Offspring were taken for study at 1–9 months of age. Homozygous *weaver* animals were identified behaviourally by their severe ataxia, hypotonia, and fine tremor⁸. Heterozygote littermates have no such obvious behavioural abnormalities but do have moderate cerebellar atrophy and were identified by retrospective examination of the cerebellum^{11,12}. Homozygous (*+wv*) littermates have not yet been examined.

For the biochemistry, whole-region dissections of the caudoputamen, nucleus accumbens, olfactory tubercle and mid-brain were made from 420- μ m-thick transverse slices cut from 17 *weaver* and 17 control brains hardened *in vivo* by transcardial perfusion with ice-cold phosphate-buffered saline (0.1 M PO₄, 0.9% saline) under conditions of barbiturate (Nembutal) anaesthesia¹³ (see Fig. 1). Whole-retina samples were also taken from some animals, so that the values for dopamine in the basal ganglia could be compared with those of the dopamine-containing amacrine cell population in the retina. Catecholamines were extracted from the isolated brain regions and retinas by homogenization in 0.1 M HClO₄. The dopamine in the supernatant was isolated using alumina mini-columns and was measured by electrochemical detection following separation by high performance liquid chromatography¹⁴. The protein content of the precipitate was determined by the method of Lowry *et al.*¹⁵.

For the anatomy, 30 μ m-thick sections were cut in a cryostat from frozen unfixed brains (13 *weavers*, 14 littermate controls), thaw-mounted onto slides and processed by de la Torre's glyoxylic acid method¹⁶ for the visualization of catecholamine-induced fluorescence. Sections were viewed and photographed under fluorescent illumination with the aid of a Leitz Ploemopak.

Table 1 summarizes the biochemical findings for all mice studied. In the controls, the concentrations of endogenous dopamine extracted from the caudoputamen, nucleus accumbens and olfactory tubercle were similar, though values for the caudoputamen were slightly higher than those for the ventral subdivisions. Dopamine levels for all three striatal districts were, as expected¹⁷, high (>200 pmol per mg protein) relative to values for the midbrain and retina (~7 pmol per mg protein). Measurements of samples from the *weaver* mice confirmed that there are reduced levels of dopamine in the striatum of this mutant¹⁰ and further demonstrated that the defect is neither shared by all dopaminergic neurons in the central nervous system nor equivalent in all regions that are affected. First, the dopamine content of the *weaver* retinas was indistinguishable from that of control retinas, suggesting that the dopamine-containing amacrine cells are normal in these mutants. Second, there was a remarkable non-uniformity in the depletion of

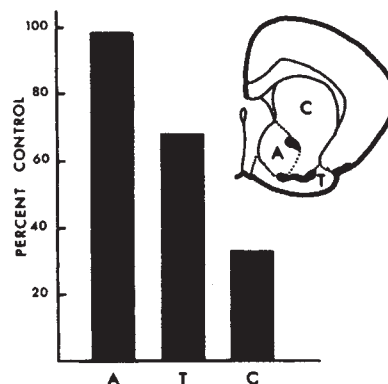


Fig. 1 The bar graph summarizes measurements, expressed as % of littermate control values, of the concentrations of endogenous dopamine extracted from the nucleus accumbens (A), olfactory tubercle (T) and caudoputamen (C) of *weaver* mice. Thirty-four mice (17 *weaver*, 17 control) were used. All brain samples were obtained from mice anaesthetized with sodium pentobarbital (Nembutal, 40 mg per kg) and perfused through the heart with chilled phosphate-buffered saline¹³. The cold-hardened brains were removed and placed on the stage of a tissue sectioner, were covered with agar and were cut into a series of 420- μ m-thick transverse slices. The face of each slice was viewed through a dissecting microscope with indirect illumination from below so that nuclei and fibre tracts could be distinguished by their differential translucency. Whole-region dissections of the caudoputamen, nucleus accumbens and olfactory tubercle were made by removing the structure from each level at which it appeared with fine cold blades. The inset to the right of the bar graph shows the borders followed in removing the three striatal districts from a representative slice. Samples containing the caudoputamen, nucleus accumbens and olfactory tubercle were homogenized in 0.1 M perchloric acid. After centrifugation, the dopamine in the supernatant was isolated on alumina mini-columns, separated by high performance liquid chromatography and measured electrochemically¹⁴. The protein content of the precipitate was determined by the method of Lowry *et al.*¹⁵. Fourteen of the *weaver*-littermate pairs were between 1 and 4 months of age. Three pairs were 6–9 months old. As no differences between the young and older animals were found, values for all ages are shown together. See Table 1 for values expressed as pmol dopamine per mg protein.

dopamine in the *weaver* striatum, with by far the largest reduction in dopamine levels appearing in the dorsal striatum.

Figure 1 summarizes the differential loss of dopamine in the striatum and shows an example of the boundaries followed in separating the three striatal subdivisions studied. The most striking contrast was between the concentrations of dopamine in the caudoputamen and in the nucleus accumbens: there was a 67% loss of dopamine in the *weaver* caudoputamen relative to control values, but virtually complete sparing of dopamine in the *weaver* nucleus accumbens. The olfactory tubercle showed an intermediate effect (32% loss). This differential pattern of biochemical abnormality was already established in the 1 month-old *weavers* and appeared unchanged in the oldest animals studied (9 months). As shown in Table 1, the dopamine concentration in the *weaver* midbrain was also abnormal, being reduced 31% relative to the control values at all ages sampled in the 1 to 9 month interval.

Measurements of protein made in *weaver* and in control mice (Table 1) point to a slight but consistent atrophy of the *weaver* caudoputamen, where protein content was reduced by 15%. By contrast, the protein concentrations measured for the nucleus accumbens and olfactory tubercle, and for the midbrain, were normal, indicating that the volumes of these regions were unaffected in the mutants. These results suggest that the striatal abnormality in the *weaver* mouse includes a component of tissue loss and that this, like the defect in dopamine, is expressed most strongly in the dorsal striatum.

The neurotransmitter defect in the *weaver* striatum was vividly demonstrated in the histofluorescence sections prepared

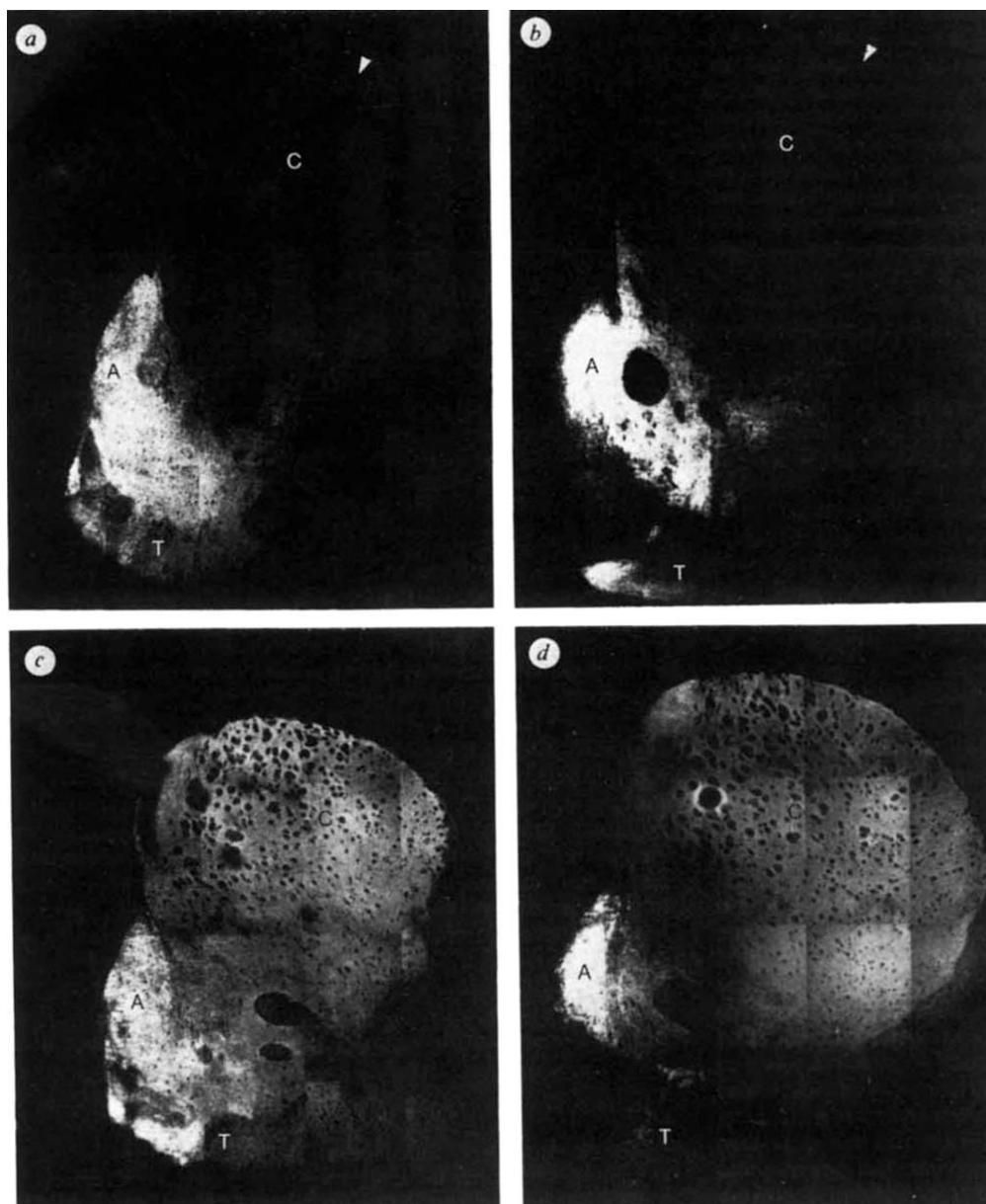


Fig. 2 Montage-photographs illustrating catecholamine-induced fluorescence (white) in the striatal complex of a 1-month-old homozygous weaver mutant mouse (*a, b*) and its heterozygous littermate control (*c, d*). Rostral cross-sections (A and C) are at midlevels of the nucleus accumbens (A). Caudal cross-sections (B and D) are near caudal tip of nucleus accumbens and olfactory tubercle (T). The sections from the control brain show the bright fluorescence distinguishing the entire striatal complex from surrounding tissue and the brilliant fluorescence in nucleus accumbens and medial olfactory tubercle. By contrast, in the sections from the weaver mutant, fluorescence is very weak in the caudoputamen (C) but appears normal in the nucleus accumbens and probably also the medial part of the olfactory tubercle. Note residual fluorescence in most ventral caudoputamen, which is considered together with the nucleus accumbens and olfactory tubercle as part of the ventral striatum.

for the anatomical experiments. This is evident in the montage-photographs of Fig. 2, which illustrate transverse sections through two levels of the striatum in a 1 month-old weaver mouse and in its littermate control. In the control brain (Fig. 2*c* and *d*) the catecholamine-induced fluorescence is brilliant in the nucleus accumbens and medial olfactory tubercle and bright throughout the rest of the striatum. As a result, the non-dopaminergic fibre bundles piercing the striatal tissue stand out as dark against the neuropil. The intensity of fluorescence varies somewhat within the caudoputamen and markedly within the nucleus accumbens and olfactory tubercle. Some of this heterogeneity almost certainly reflects local differences in innervation density or type, especially in the accumbens and olfactory tubercle. The details of the patterning varied from section to section.

In the weaver brain (Fig. 2*a, b*) the entire dorsal part of the caudoputamen is darkened. The fluorescence is not completely missing in this region, because there still is a discriminable difference in brightness between striatal and non-striatal tissue (see arrows). Even so, compared to that in the littermate control, the fluorescence clearly is very weak dorsally and hardly visible dorsolaterally. By contrast, in the most ventral part of the caudoputamen, below the depth-level of the anterior commissure, the intensity of fluorescence approaches control levels, and

both the nucleus accumbens and the medial part of the olfactory tubercle show a brilliant apparently normal fluorescence. In the nucleus accumbens of the weaver, the degree of heterogeneity in the distribution of fluorescence resembles that in the control (contrast Fig. 2*a, c*). The relative brightness in at least the medial part of the olfactory tubercle also seems similar.

A similar loss of fluorescence in the caudoputamen and sparing of fluorescence in the nucleus accumbens and medial olfactory tubercle was observed in all the weaver brains examined and in each, a gradient of loss was evident in the caudoputamen, the least dimming of fluorescence being ventral. The patterns in the olfactory tubercle were more variable, for although the medial part of the tubercle always seemed to be spared, fluorescence laterally was more difficult to see in some weaver brains than in others. Variability may partly have been associated with edge artefacts produced at the time of sectioning.

To a remarkable degree, the topography of dopamine loss in the weaver striatum respects the subdivisions of the striatum drawn on the basis of its nigrostriatal and mesolimbic inputs and represented in the terms dorsal (non-limbic) and ventral (limbic) striatum. It is particularly striking that in the weaver mouse, the region of remaining fluorescence exceeds the nucleus accumbens and medial olfactory tubercle to include the ventral part of the caudoputamen, just as the mesolimbic system (as

Table 1 Content of dopamine and protein in three striatal regions, midbrain and retina of weaver and littermate control mice

Region	Genotype	Dopamine		Protein	
		(pmol per mg protein)	% Control	(mg)	% Control
Striatum					
Caudoputamen	+/ <i>wv</i>	272 ± 18 (21)		1.63 ± 0.02	
	<i>wv/wv</i>	89 ± 4 (23)	33*	1.39 ± 0.03	85*
N. accumbens	+/ <i>wv</i>	242 ± 13 (26)		0.104 ± 0.005	
	<i>wv/wv</i>	238 ± 11 (25)	98	0.112 ± 0.006	108
Olfactory tubercle	+/ <i>wv</i>	228 ± 10 (6)		0.162 ± 0.006	
	<i>wv/wv</i>	156 ± 10 (8)	68*	0.155 ± 0.008	96
Midbrain					
	+/ <i>wv</i>	7.3 ± 0.4 (11)		5.36 ± 0.34	
	<i>wv/wv</i>	5.1 ± 0.3 (10)	69*	5.00 ± 0.26	93
Retina					
	+/ <i>wv</i>	7.0 ± 0.63 (11)		0.328 ± 0.009	
	<i>wv/wv</i>	7.9 ± 0.53 (10)	113	0.343 ± 0.013	105

Extractions and measurements of dopamine and protein were made following whole-region dissections of striatum and midbrain as described for Fig. 1. The retina was dissected with the aid of a microscope after removing the eye and bisecting the retina with sharp blades inserted into the optic stalk. A total of 17 control and 17 weaver mice were used but not all regions were examined in each animal. For the three divisions of striatum, dopamine content was measured separately in the right and left hemispheres. There were no left-right differences, thus dopamine and protein values from right and left are combined in the table. Animals ranged in age from 1 to 9 months with most being under 4 months of age. All regions were examined in both young and old animals. No differences were detected between younger and older animals, and thus data from all animals were pooled. Values shown are mean ± standard error of the mean. The numbers in parentheses represent numbers of samples. Statistical comparisons were conducted with Student's two tailed t-test. * $P < 0.001$.

charted in the rat) projects to the ventral caudoputamen in addition to the accumbens-tubercle proper¹⁸⁻²¹. Although a detailed mapping is still needed, we conclude that the weaver disease affects dopamine levels mostly in the dorsal striatum and spares dopamine mostly in the ventral striatum. This suggestion is strengthened by our preliminary observations on the distribution of tyrosine hydroxylase-like immunoreactivity in the weavers, which also shows a sharp depletion dorsally (manuscript in preparation).

These findings strongly suggest that the nigrostriatal and mesolimbic dopamine systems are differentially vulnerable in genetic disorders. Just how the weaver mutation affects the dopamine-containing neurons in the midbrain, however, is not yet clear. In the cerebellum, the extensive death of granule cells in the early postnatal period is thought to result from a failure of granule cells to migrate from their proliferative zone²². Most evidence shows the migratory defect to be a consequence of a direct action of the weaver gene on the granule cells²³⁻²⁶ but the possibility of a direct effect on the Bergmann glial guide fibres has not been excluded^{24,25}. Schmidt *et al.*¹⁰ have suggested that the dopamine defect in weaver is also developmental because striatal dopamine levels are nearly normal in neonatal weavers but fail to increase with age. These authors describe cell loss in the adult weaver substantia nigra pars compacta, but report normal concentrations of dopamine in the brainstem. Our own work shows that the concentration of dopamine in the weaver midbrain is decreased by about one-third (31%) relative to control values (Table 1). In the anatomical material, fluorescing neurones can be detected in each of the midbrain dopaminergic cell groups, including the pars compacta of the substantia nigra, but results from a quantitative study, now under way, will be needed to determine whether a particular subpopulation of dopaminergic neurones is missing in the weaver. Given the pattern of striatal loss, an abnormality of neurones in the substantia nigra's pars compacta would clearly be most likely.

Plausible alternatives to cell loss include not only metabolic defects but also abnormality of certain axon-collateral systems, as has been suggested for the mutant mouse tottering²⁷. Any of these factors could reflect a primary action of the weaver gene on midbrain neurones and glia (for example, on cells migrating at a particular time) or could represent effects secondary to changes in the forebrain (for example, in response to specific defects in target cells or receptors). The possibility of a differential loss of dopamine receptors in the dorsal striatum deserves special emphasis because Boehme and Ciaranello²⁸ have shown that strain differences exist in striatal dopamine receptor binding in inbred mice, and that there is independent genetic control of

receptors in the olfactory tubercle and more dorsal striatum. A study of the distribution of dopamine receptors in weaver is clearly needed. Strain differences in the numbers of dopamine-containing neurones in the midbrain and in corresponding differences in midbrain tyrosine hydroxylase activity²⁹ have also been reported, but apparently the effects are similar in the substantia nigra and ventral tegmental area³⁰.

A general implication of the present findings is that genetic control of dopaminergic systems in the forebrain may be exerted separately on subunits defined in functional³¹, pharmacological³², and anatomical¹ terms. Of the parts of the dopamine-recipient forebrain so far analysed in weaver, the nucleus accumbens is singled out as being fully spared a dopamine deficit, the dorsolateral caudoputamen as being most severely affected. No hereditary disease in the human has yet been shown to affect different parts of the striatal dopamine mechanism with such selectivity. The findings in weaver point to the potential for such dissociations, however, and this is of interest given the suspected differential contributions of dopaminergic fibres projecting to the dorsal and ventral striatum in modulating the organization of movement patterns and their motivational contexts³¹. In this regard it is striking that in Huntington's disease, an autosomal dominant disorder in which the striatum atrophies, neurones in the nucleus accumbens are spared relative to those in the caudoputamen³³ just as the content of dopamine and of protein in the weaver nucleus accumbens is spared relative to that in the weaver caudoputamen. The weaver disorder might thus hold clues to general patterns of tissue and neurochemical loss produced in the striatum in inherited extrapyramidal diseases.

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1. Ungerstedt, U., *Acta physiol. scand.* **82**, Suppl. 367, 1-48 (1971).
2. Thierry, A. M., Blanc, G., Sobel, A., Stinus, L. & Glowinski, J. *Science* **182**, 499-501 (1973).
3. Lindvall, O., Björklund, A., Moore, R. Y. & Stenevi, U. *Brain Res.* **81**, 325-331 (1974).
4. Hornykiewicz, O. *Wiener Klinische Wochenschrift* **75**, 309-312 (1963).
5. Stevens, J. R. *Arch. Gen. Psychiatry* **29**, 177-189 (1973).
6. Iversen, L. L. *Science* **199**, 1084-1089 (1975).
7. Mackay, A. V. P., Iversen, L. L., Rossor, M., Spokes, E., Bird, E., Arregui, A., Creese, I. & Snyder, S. H. *Arch. Gen. Psychiatry* **39**, 991-997 (1982).
8. Sidman, R. L. in *Physiological and Biochemical Aspects of Nervous Integration* (ed. Carlson, F. D.) 164-193 (Prentice-Hall, Inc., Englewood Cliffs, N.J., 1968).
9. Lane, J. D., Nadi, N. S., McBride, W. J., Aprison, M. H. & Kusano, K. *J. Neurochem.* **29**, 349-350 (1977).

10. Schmidt, M. J., Sawyer, B. D., Perry, K. W., Fuller, R. W., Foreman, M. M. & Ghetti, B. *J. Neurosci.* **2**, 376–380 (1982).
11. Rakic, P. & Sidman, R. L. *J. Comp. Neurol.* **152**, 103–132 (1973).
12. Roffler-Tarlov, S. & Turey, M. *Brain Res.* **247**, 65–73 (1982).
13. Zigmond, R. & Ben-Ari, Y. *J. Neurochem.* **26**, 1285–1287 (1976).
14. Moyer, T. P. & Jiang, N.-S. *J. Chromatography* **153**, 365–372 (1978).
15. Lowry, O. H., Rosebrough, N. I., Farr, A. L. & Randall, R. J. *J. Biol. Chem.* **193**, 265–275 (1951).
16. de la Torre, J. C. *J. Neurosci. Methods* **3**, 1–5 (1980).
17. Versteeg, D. H. G., Van der Gugten, De Jong, W. & Palkovits, M. *Brain Res.* **113**, 563–574 (1976).
18. Fallon, J. H. & Moore, R. Y. *J. Comp. Neurol.* **180**, 545–580 (1978).
19. Kelley, A. E., Domesick, V. B. & Nauta, W. J. H. *Neuroscience* **7**, 615–630 (1982).
20. Heimer, L. & Wilson, R. D. in *Golgi Centennial Symposium* (ed. Santini, M.) 177–193 (Raven Press, New York, 1975).
21. Beckstead, R. M., Domesick, V. B. & Nauta, W. J. H. *Brain Res.* **175**, 191–217 (1979).
22. Rezaei, Z. & Yoon, C. H. *Develop. Biol.* **29**, 17–26 (1972).
23. Sotelo, C. & Changeaux, J.-P. *Brain Res.* **77**, 484–491 (1974).
24. Goldowitz, D. & Mullen, R. J. *J. Neurosci.* **2**, 1474–1485 (1982).
25. Hatten, M. E., Liem, R. K. H., Shelanski, M. L. & Mason, C. A. *Society for Neuroscience Abstract*, 63.21 (1982).
26. Willinger, M., Margolis, D. M. & Sidman, R. L. *J. Supramol. Struct. and Cell. Biochem.* **17**, 79–86 (1981).
27. Levitt, P. & Noebels, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4630–4634 (1981).
28. Boehme, R. E. & Ciaranello, R. D. *Brain Res.* **266**, 51–65 (1983).
29. Ross, R. A., Judd, A. B., Pickel, V. M., Joh, T. M. & Reis, D. J. *Nature* **264**, 654–656 (1976).
30. Baker, H., Joh, T. H. & Reis, D. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4369–4373 (1980).
31. Iversen, S. D. & Fray, P. J. in *The Neural Basis of Behavior* 229–269 (Spectrum Publications, Inc., 1982).
32. Fuxe, K., Fredholm, B. B., Agnati, L. F. & Corrodi, H. *Brain Res.* **146**, 295–311 (1978).
33. von Sattel, J.-P., Ferrante, R. J. & Richardson, E. P. *J. Neuropath. and Exp. Neurol.* submitted.
34. Graybiel, A. M. & Ragsdale, C. W. in *Development and Chemical Specificity of Neurons* (eds Cuénod, M., Kreutzberg, G. W. & Bloom, F. E.) 239–283 (Elsevier, Amsterdam, 1979).

Adrenal chromaffin cells form functional cholinergic synapses in culture

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Adrenomedullary cells and autonomic ganglion cells originate from the neural crest. Both cell types synthesize, store and release catecholamines; however, their structural and functional properties are distinctly different. Aloe and Levi-Montalcini¹ have shown *in vivo* that when the adrenal medulla is exposed to exogenous nerve growth factor (NGF) most cells differentiate into neuronal cells substantially similar to sympathetic neurones. Experiments *in vitro*^{2–7} have also shown that neonatal as well as adult adrenal chromaffin cells and their neoplastic correlate (PC12 cells) undergo neurone-like morphologic differentiation in response to NGF. From these morphological and biochemical studies alone, however, it remains uncertain whether the functional neuronal transformation is also accompanied. We report here that the adrenal chromaffin cells in culture can differentiate into neuronal cells having functional synapses which were found to be cholinergic in nature. Furthermore, the type of synaptic vesicles in the newly formed synapses was apparently dependent upon K⁺ levels in the culture medium.

Adrenal glands were removed aseptically from 8- to 10-day-old Wistar strain rats of either sex. Medullae were carefully freed from capsular and cortical tissue by using a pair of knives and/or fine tungsten needles under a dissecting microscope, and then incubated with dispase (1,000 U per ml culture medium; Godo Shusei) for 1 h at 36 °C. After washing by centrifugation and resuspension, the cells were seeded onto 35 mm Falcon dishes containing a control culture medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 20%

fetal calf serum). Cultures were maintained at 37 °C in a water-saturated atmosphere of 95% air and 5% CO₂; the medium was changed every 2 to 3 days. A fraction of 2.5S NGF, prepared from the submandibular glands of adult male mice⁸, was added at a concentration of 10–100 ng per ml of medium throughout the culture period. Glass micropipettes filled with 4 M potassium acetate (electrode resistance, 20–60 MΩ) were used for both recording membrane potential and passing current by means of a high input-impedance amplifier with the bridge circuit.

As regards purity of the cell populations thus prepared, an exhaustive, phase-microscopic surveillance was routinely made at least twice (on days 3 and 6 of culture) for every preparation of medullary cells; this was possible because of the relatively low density of the cells plated (1–3 × 10³ cells per dish). The surveillance revealed that the maximum contamination by non-chromaffin, polygonal, neuronal cells having large nuclei with conspicuous nucleoli was 0.3% (at most 3 neuronal cells per dish), a result in good agreement with Unsicker *et al.*², who reported a value of 0.25% (~50/20,000) for the neuronal contamination. This value, together with histochemical evidence (Fig. 1B, C), ensures the present study from the possible risk of selectively impaling the originally neuronal cells. This latter risk could also be evaded by an omission of NGF from the early stage of cell culture because under that condition neuronal contaminants, if any, would not have survived⁹. The rat adrenal chromaffin cells, as shown with human chromaffin cells⁴, survived more than 20 days in culture without NGF. Cultures to which NGF was added during the second week contained approximately the same number and percentage of process-forming cells as cultures in which NGF was added at the beginning of the culture period. The only difference was that cholinergic synaptic interactions were first detected around 20 days (see below).

Shortly after the individual or small groups of cells had settled down onto the dish surface, the initially dispersed chromaffin cells showed a tendency to adhere to each other to form numerous large cell clusters, and fine unmyelinated processes concomitantly began to grow out from the cell soma. Such processes continued to grow and the final dense networks of neurites were usually formed after 2 weeks in culture (Fig. 1A). Fluorescence cytochemistry similar to that used by Unsicker *et al.*^{2,3}, revealed that almost all of the clustered cells at this stage still retained the chromaffin nature as judged from their strong catecholamine fluorescence (Fig. 1B, C). Around the end of 2 weeks or later, examination under the electron microscope (Fig. 1D) revealed the formation of some synaptic structures or varicosities that contained abundant clustered small clear vesicles (40–50 nm in diameter) and a few large dense-cored vesicles (~90 nm). The former vesicles are similar in size to vesicles usually observed in the synapses of cultured sympathetic neurones^{10,11}. Note also the occurrence of intermediate organelles in cells at this time (Fig. 1E). There are now pleomorphic dense-cored vesicles. These may reflect the transition from a typical chromaffin granule inclusion to the neuronal synaptic organelles, including round electron-lucent and dense-cored vesicles.

Synaptic interaction between the chromaffin cells was tested by impaling, with microelectrodes under visual control, pairs of cells randomly selected from two neighbouring clusters. As shown in Fig. 2A, intracellular stimulation of cell 2 (c2) elicited an excitatory postsynaptic potential (e.p.s.p.) in cell 1 (c1) with a delay of 3.7 ms, which, in turn, occasionally triggered an action potential. When examined in a pair of nearest clusters of 4 weeks culture, such evoked e.p.s.ps were recorded from 36 pairs of cells out of 50, including 17 pairs which exhibited 'reciprocal' synaptic interaction. This observation also supports our contention that the events described here are associated with the chromaffin cells but not with the neuronal contaminants. The following observations indicate that the synaptic transmission involved was chemically mediated: (1) a delay of a few milliseconds between firing in the presynaptic cell and depolarization in the postsynaptic cell, (2) no passive transfer of hyperpolarizing

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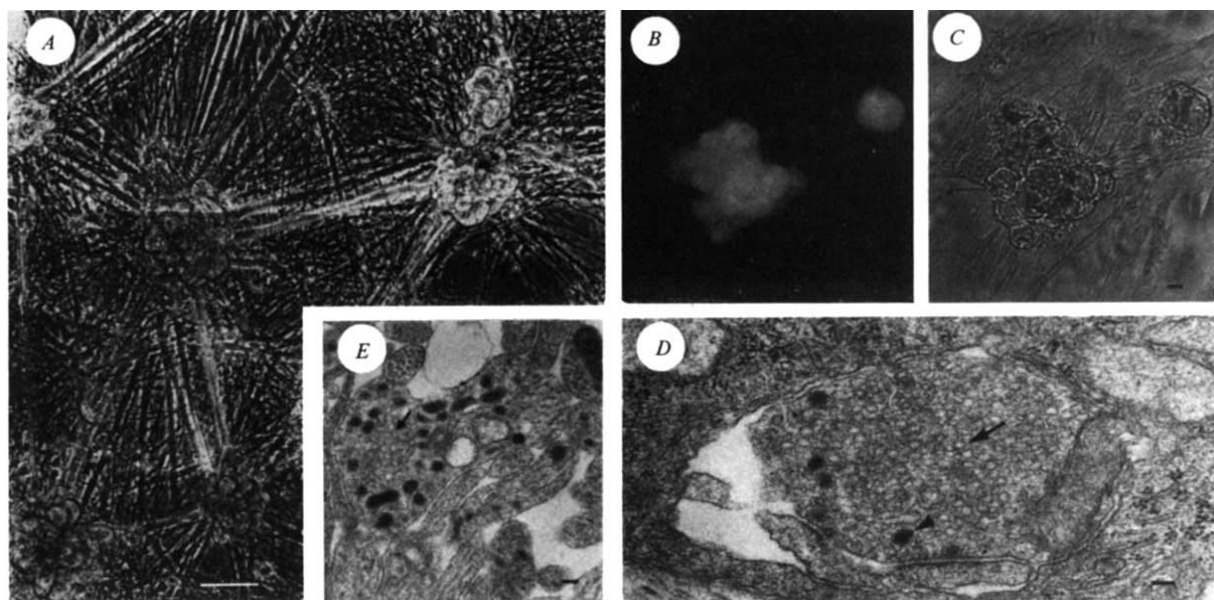


Fig. 1 **A**, Phase-contrast micrograph of chromaffin cells cultured with NGF for 30 days. The cells, immediately after dissociation, assumed a round shape with no stumps of processes. In 1 week of culture, many of the isolated chromaffin cells tended to gather into numerous clusters, from which fine fibrous processes extended. In 2 to 3 weeks, contaminating fibroblasts increased to confluency, while the clustered chromaffin cells built up a complex network of straight fibres interconnecting the clusters. In this micrograph, fibroblastic proliferation was substantially suppressed by treatment with cytosine arabinoside ($10 \mu\text{M}$) over days 21 and 22 after plating. Scale bar, $100 \mu\text{m}$. **B**, Glyoxylic acid-induced catecholamine fluorescence¹⁴ of the clustered cells after 2 weeks in culture. The same cells can be identified in **C**. In some cases catecholamine-specific fluorescence, though weaker than that of the cell soma, was also detected from neurite extensions (not shown). **C**, Light micrograph of the field in **B**. Scale bar, $10 \mu\text{m}$. **D**, Electron micrograph of a synaptic structure formed between the cultured chromaffin cells 35 days after plating. Note the synaptic membrane thickening of increased density and the accumulation of small clear vesicles (arrow); a few large dense-core vesicles having an electron-lucent halo around the core (arrowhead) are also present. The specimen was fixed in phosphate buffered 2% glutaraldehyde ($\text{pH } 7.2$) followed by buffered 1% osmium tetroxide, dehydrated through graded ethanol, and then embedded in Spurr's epoxy resin¹⁵. Scale bar, 100 nm . **E**, Electron micrograph of an intermediate cell type of chromaffin cells cultured for 2 weeks. Small clear vesicles (arrow) and pleomorphic dense-cored vesicles are present. Preparation method similar to **D**. Scale bar, 100 nm .

or subthreshold depolarizing pulses between the test cells, (3) the amplitude of e.p.s.ps varied from one presynaptic stimulus to another, (4) the amplitude of e.p.s.ps was sensitive to the level of postsynaptic membrane potential, increasing with hyperpolarization, and (5) the amplitude of e.p.s.ps increased with an increase in the extracellular Ca^{2+} concentration.

In an attempt to identify the transmitter type responsible for the chemical transmission, we applied several cholinergic and adrenergic blocking agents over the cells that demonstrated synaptic interaction. As shown in Fig. 2, the e.p.s.ps were reversibly affected by (+)tubocurarine (TC), a cholinergic blocker, and also by hexamethonium (data not shown). In contrast, none of these synaptic responses was affected by phentolamine, an α -adrenergic blocker, even at 0.1 mM . The chromaffin cells usually showed spontaneous synaptic potentials after 2 weeks in culture (Fig. 3). The cell illustrated in Fig. 3A exhibited spontaneous e.p.s.ps, summation of which gave rise to action potentials. The time course of these responses was similar to that observed in the evoked synaptic responses. These spontaneous e.p.s.ps were also abolished by the cholinergic blocking agent (Fig. 3B).

The above data collectively indicate that the adrenal chromaffin cells can develop several neuronal characteristics and form cholinergic synapses between them. This finding raises the possibility that the cultured chromaffin cells may also be plastic with respect to the transmitter choice as reported for the sympathetic ganglion cells, in which the culture environment is known to determine the neurotransmitter selection (see ref. 12 for review). In fact, it has been shown that elevated K^+ concentrations in culture medium prevent the conversion of the superior cervical ganglion cells from an adrenergic to a cholinergic phenotype¹³. When we cultured the chromaffin cells in an NGF-containing high K^+ medium, which was made by diluting Dulbecco's minimal essential medium (DMEM) with 0.2 M KCl

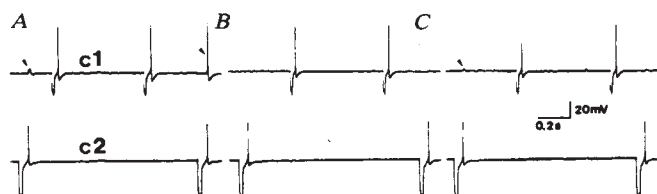


Fig. 2 Intracellular recording of membrane potentials and e.p.s.ps from the chromaffin cells demonstrating chemical synaptic transmission after 32 days culture. Test cells 1 and 2 (c1 and c2), separately chosen from a pair of neighbouring clusters, were stimulated asynchronously by anodal break excitation. Traces **A**, **B** and **C** are all from the same paired cells. For electrophysiological experiments, the culture dish was placed on an inverted phase-microscope stage thermostated at $33\text{--}35^\circ\text{C}$ by circulating water. The test dish was perfused, at about 25 ml h^{-1} , with a prewarmed control solution of Eagle's medium containing 5 mM HEPES ($\text{pH } 7.4$). **A**, Anode-break excitation in c2 evoked sub- or supra-threshold e.p.s.p in c1 (arrowheads) with a delay of 3.7 ms ; however, firings of c1 had no such effect on c2, indicating that a synapse mechanism was involved. **B**, Perfusion with TC ($25 \mu\text{M}$) abolished the synaptic response. No change was observed in the anode-break action potentials or in the resting levels of both cells. **C**, After 10 min perfusion with the control solution the cells resumed synaptic activity.

to yield $\sim 20 \text{ mM K}^+$, the cells showed a progressive elongation of processes and formed dense neurite networks such as shown in Fig. 1A. Spontaneous or evoked synaptic activity, however, was detected in none of more than 100 cells examined in over 25 culture sets of the high K^+ medium (Fig. 3C). On the other hand, the resting potentials of these cells were in the normal range, and the cells were able to produce action potentials.

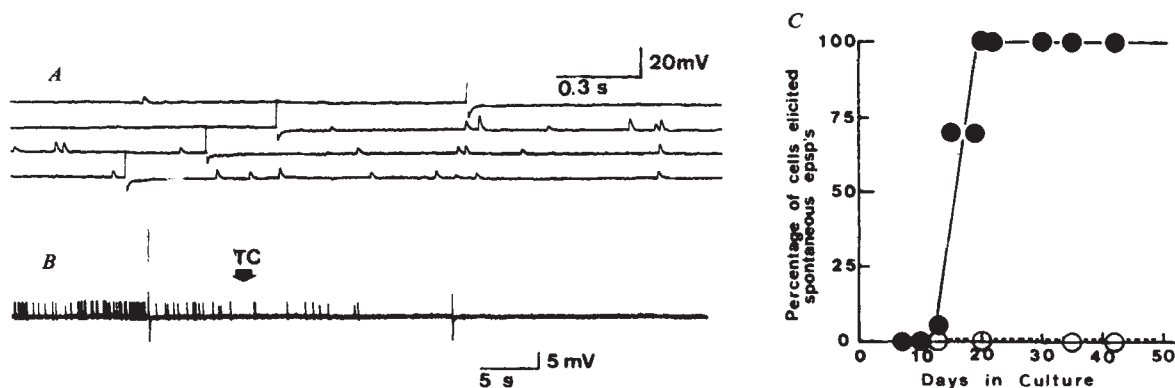


Fig. 3 *A*, Spontaneous synaptic activity recorded from a chromaffin cell cultured for 35 days. All traces are from one continuous record. *B*, Spontaneous e.p.s.ps were also sensitive to TC (25 μM). *C*, Development of spontaneous e.p.s.ps from cells cultured in normal K⁺ medium (●) and no such occurrence of e.p.s.ps in high K⁺ cultures (○). In each case, 20 cells randomly sampled from 10 different clusters were impaled by a microelectrode with its tip held inside the respective test cells for at least 5 min. Each point represents the percentage of successful detection of spontaneous e.p.s.ps. Test medium was Eagle's minimal essential medium containing 5.3 mM K⁺. Test cells were all from the same preparation.

Sister cultures with the normal K⁺ medium (DMEM diluted with 0.2 M NaCl instead of 0.2 M KCl) showed the same pattern of synaptic activities as in the control culture. The loss of synaptic activity in the cells cultured in the high K⁺ medium is not due

to the absence of sensitivity to acetylcholine (ACh), since the responses to ACh iontophoresis (as high as 400 mV nC⁻¹) were indistinguishable from those observed in the control cells. The chromaffin cells, cultured in the high or the normal K⁺ medium, were not responsive to adrenaline and noradrenaline.

To determine whether the high K⁺ condition modulates the transmitter choice towards the enhanced adrenergic selection, the population of synaptic vesicles was examined ultrastructurally using the KMnO₄-fixation procedure, a well known technique to identify the adrenergic vesicles^{10,11}. As shown in Fig. 4*B*, most of the synaptic vesicles found in the high K⁺ culture were granular, a fact indicative of adrenergic vesicles. In the normal K⁺ cultures, however, synapses and varicosities contained over 60% clear vesicles (Fig. 4*A*). Such an observation implies that, in the high K⁺ medium, the chromaffin cells may form predominantly adrenergic synapses, and yet the adrenergic synaptic activity failed to be detected electrophysiologically because of a lack of postsynaptic receptors for catecholamines.

Finally, our observation that the adrenal chromaffin cells from neonatal rats can be transformed into neuronal cells equipped with the functional synapses of cholinergic or adrenergic nature depending on the culture condition, supports the view that the precursors of the adrenal and the sympathetic neuronal cells are pluripotent at a certain stage in development and then differentiate into their respective phenotypes under the control of some environmental factors.

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1. Aloe, L. & Levi-Montalcini, R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1246-1250 (1979).
2. Unsicker, K., Krusch, B., Otten, U. & Thoenen, H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3498-3502 (1978).
3. Unsicker, K. & Chamley, J. H. *Cell Tissue Res.* **177**, 247-268 (1976).
4. Tischler, A. S., De Lellis, R. A., Biales, B., Nunnemacher, G., Garraba, V. & Wolfe, H. J. *Lab. Invest.* **43**, 399-409 (1980).
5. Naujoks, K. W., Korshing, S., Rohrer, H. & Thoenen, H. *Dev. Biol.* **92**, 365-379 (1982).
6. Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2424-2428 (1977).
7. Doupe, A. J., Patterson, P. H. & Landis, S. C. *Soc. Neurosci. Abstr.* **8**, 257 (1982).
8. Bocchini, V. & Angeletti, P. V. *Proc. natn. Acad. Sci. U.S.A.* **64**, 787-794 (1969).
9. Johnson, M. I., Ross, C. D., Meyers, M., Spitznagel, E. L. & Bunge, R. P. *J. Cell Biol.* **84**, 680-691 (1980).
10. Landis, S. C. *Dev. Biol.* **77**, 349-361 (1980).
11. Chun, L. L. Y. & Patterson, P. H. *J. Cell Biol.* **75**, 694-704 (1977).
12. Patterson, P. H. *A. Rev. Neurosci.* **1**, 1-17 (1978).
13. Walicke, P. A., Campenot, R. B. & Patterson, P. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5767-5771 (1977).
14. De la Torre, J. C. & Surgeon, J. W. *Histochemistry* **49**, 81-93 (1976).
15. Spurr, A. R. *J. ultrastruct. Res.* **26**, 31-43 (1969).

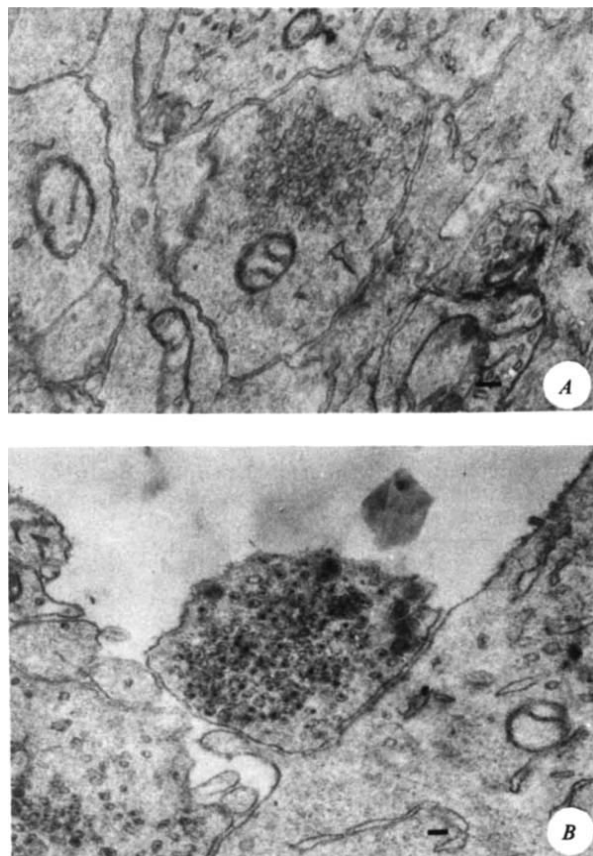


Fig. 4 Electron micrographs (KMnO₄ fixation) of synaptic vesicles contained in the chromaffin cells that were cultured with NGF for 30 days in: *A*, normal K⁺ medium, and *B*, high K⁺ medium. The cells were preincubated for 30 min at 37°C with 10⁻⁵ M noradrenaline freshly dissolved in DMEM, washed in Ringer solution, and then fixed in KMnO₄ (3%, in Ringer) at 4°C for 1 h. After washing the cells were stained *en bloc* by uranyl acetate (1%, in 0.1 M nabic acid buffer, pH 5.2) at 4°C for 1-2 h. Dehydration and embedding were processed as in legend to Fig. 1*D*. Scale bar 100 nm.

Slow transmission of neural activity in hippocampal area CA1 in absence of active chemical synapses

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Transmission of neural activity in the mammalian cortex involves the operation of chemical synapses^{1,2}. Here we report that activity of hippocampal pyramidal cells (HPC) *in vitro* can spread through the neural aggregate even when chemical synaptic transmission is blocked by lowering the external Ca^{2+} concentration ($[\text{Ca}^{2+}]_o$). In these conditions focal stimulation of the HPC body layer (stratum pyramidale) at area CA1 evokes a localized repetitive neural response which, when large enough, spreads along this layer in both transverse directions. It was recently reported for similar conditions^{3,4} that the activated HPC often discharge in synchrony. However, the spread of the neural response does not depend upon the occurrence of synchronized HPC activity, and therefore represents a distinct phenomenon.

Several lines of evidence suggest that K^+ accumulation in the extracellular space is responsible for the transmission of neural activity through area CA1 in conditions of blocked chemical synapses. (1) The mean velocity of spread was 1.7 mm s^{-1} , which probably is too slow to be mediated by electrical coupling or ephaptic interactions, but is compatible with K^+ redistribution by a spatial buffer mechanism⁵. (2) A rise in $[\text{K}^+]_o$ precedes and accompanies the spread of the neural response. (3) Focal applications of K^+ at the CA1 stratum pyramidale can elicit similar neural responses. (4) The spread is accelerated or slowed by increases or decreases in the baseline level of $[\text{K}^+]_o$, respectively. Such K^+ -mediated transmission of neural activity may have an important role in the spread of epileptiform discharges, which does not necessarily conform to anatomical pathways⁶, and which involves synchronous neural firing⁷ and large decreases and increases in $[\text{Ca}^{2+}]_o$ and $[\text{K}^+]_o$ ^{8,9}, respectively.

Experiments were performed on 44 rat hippocampal slices, prepared and maintained in a perfused chamber using previously described techniques^{10,11}. Changes in $[\text{Ca}^{2+}]_o$ and $[\text{K}^+]_o$ were monitored with ion-selective microelectrodes (ISM)⁸. Field potentials were recorded with either the reference barrel of the ISM or with single NaCl-filled microelectrodes. Measurements were usually obtained simultaneously from 2–4 adjacent sites (0.02–1 mm apart) in the stratum pyramidale.

To block chemical synaptic transmission, Ca^{2+} was omitted from the physiological solution (in mM: 124 NaCl, 5 KCl, 1.25 NaH_2PO_4 , 2 MgSO_4 , 2 CaCl_2 , 26 NaHCO_3 , 10 D-glucose) perfusing the slices. This resulted in a slow decrease of $[\text{Ca}^{2+}]_o$ to levels of $1\text{--}5 \times 10^{-5} \text{ M}^{11}$. During the decay of $[\text{Ca}^{2+}]_o$, the synaptically evoked responses of CA1 HPC, induced by stimulating the commissural/Schaffer collateral afferent fibres in the stratum radiatum, initially became repetitive and then gradually diminished, until they were totally blocked^{11,12}. In these conditions, however, single-shock stimuli applied at the stratum pyramidale (Fig. 1A) evoked prolonged (1–5 s), large (1–7 mV) negative potential shifts (NPS) (Fig. 1B), which were maximal in amplitude at the stratum pyramidale and smaller when recorded from the dendritic layers (strata radiatum and oriens). Recordings with K^+ -selective ISM showed that each NPS is accompanied by a transient increase (1–7 mM above baseline levels) in $[\text{K}^+]_o$ (Fig. 2), which was also maximal at the stratum pyramidale. These findings suggest that the NPS reflect the depolarization of neurones and glia induced by K^+ released

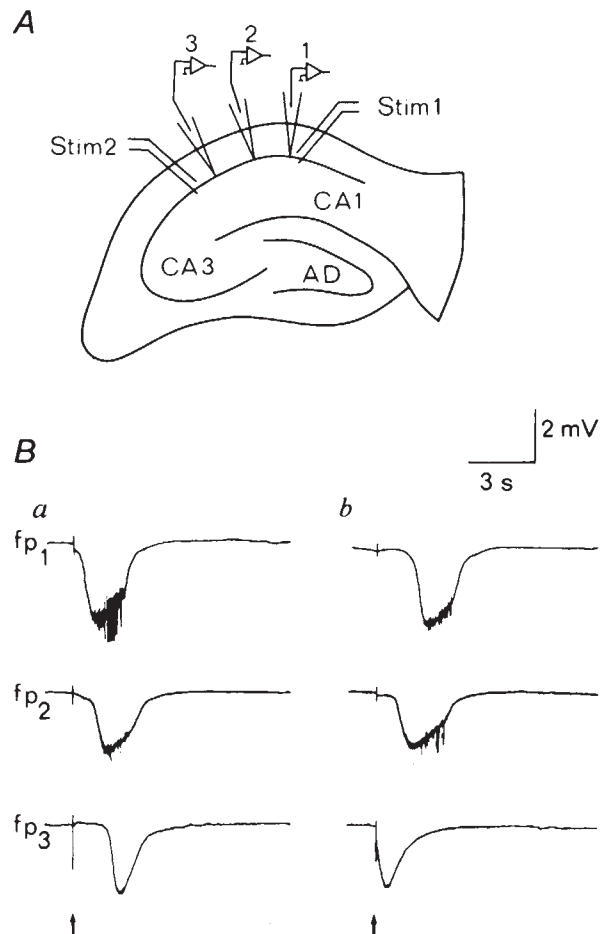


Fig. 1 Transmission of evoked neural activity along hippocampal area CA1 after blocking chemical synaptic transmission by lowering $[\text{Ca}^{2+}]_o$. **A**, The experimental arrangement. Recording microelectrodes (1–3), filled with 100 mM NaCl, were positioned 200 μm apart at the CA1 stratum pyramidale. Stimulating electrodes (Stim1, Stim2) were placed 20–50 μm from the ends of the microelectrode array. AD, area dentata. **B**, Simultaneous recordings by the three microelectrodes (1–3) of the field potentials (fp_1 , fp_2 and fp_3 , respectively), evoked in response to single-shock stimuli (70 μs , 40 nA) delivered by Stim1 (**a**) or Stim2 (**b**). Arrows indicate the stimulation artefacts. The responses, composed of a negative potential shift on top of which population spikes can be observed at two recording sites (fp_1 , fp_2), appeared about 25 min after changing from the standard solution to perfusing solution lacking Ca^{2+} . The simultaneous recordings demonstrate an increase in latency of the NPS with the distance between the recording and stimulating sites, thus suggesting a spread of neural activity along the stratum pyramidale.

from activated neurones^{3,11,13}. Indeed, extracellular single unit recordings revealed increased firing of HPC during the NPS. Furthermore, at approximately 60% of the slices, the evoked NPS were associated with bursts of population spikes (Fig. 1B, upper traces), indicating that during the NPS, the repetitive firing of individual HPC often became synchronized^{3,4}. Similar responses were also observed in slices perfused with a 0.2 mM Ca^{2+} -containing solution, in which the synaptically evoked field potentials were also completely blocked. These responses were promptly abolished by reintroducing the standard perfusing solution, whereas the normal synaptically evoked field potentials reappeared. With prolonged (>1 h) perfusion in low- Ca^{2+} solutions, NPS also appeared spontaneously in most of the slices, as has been recently described^{3,11}.

Once initiated by lowering $[\text{Ca}^{2+}]_o$, the responses described above could be evoked by stimulation anywhere along the CA1 stratum pyramidale. When the recording microelectrode was placed near (20–50 μm) the site of stimulation, the response had a brief delay of several milliseconds. This delay increased

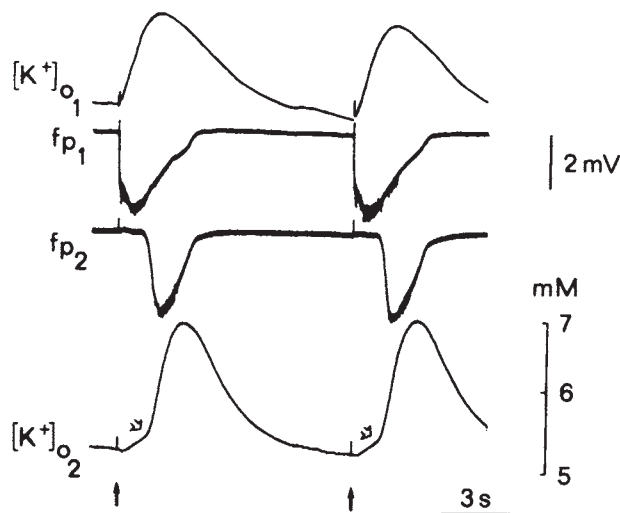


Fig. 2 Changes in $[K^+]_o$ which accompany the spread of neural activity along area CA1, in a slice with blocked chemical synaptic transmission. The experimental arrangement and procedure were basically as described in Fig. 1 legend, except that recording microelectrodes 1 and 2 are double-barrelled K^+ -ISM¹³, placed 300 μ m apart. The K^+ -selective barrel was filled with valinomycin exchanger and 100 mM KCl, and had a minimum response of 45 mV to a 10-fold change in $[K^+]_o$; the reference barrel contained 124 mM NaCl. The responses to two consecutive single-shock stimuli (filled arrows indicate stimulus artefacts) delivered by Stim1 are shown. Simultaneous recordings of the field potentials (fp_1 , fp_2) and the changes in $[K^+]_o$ ($[K^+]_{o1}$, $[K^+]_{o2}$) by ISM 1 and 2, respectively, suggest a spread of neural activity along the stratum pyramidale away from the stimulation site. Note that ISM 1 monitors a monophasic K^+ transient, whereas ISM 2 shows a biphasic increase (transition between the two phases marked by open arrows).

with the distance between the sites of stimulation and recording. With simultaneous recording from 2–4 separated sites along this layer, the response appeared as a wave of activity, which could be transmitted from the site of stimulation towards either end of area CA1 (Fig. 1B). The velocity of this spread varied in different slices from 0.44 to 4.2 $mm\ s^{-1}$ ($1.7 \pm 1.21\ mm\ s^{-1}$, mean $\pm$ s.d., $n = 11$), but in any one slice was the same in both directions. When stimuli were delivered simultaneously at both ends of area CA1 no summation of the response occurred at a recording site in between. Furthermore, several seconds following stimulation at one end of area CA1, a stimulus delivered to the other end was ineffective in eliciting a spreading response. These findings suggest that regardless of its direction of spread, the same neural population participates in the response.

In the absence of normal chemical synaptic function other factors must mediate the spread of neural activity in area CA1. Possible mechanisms are electrical coupling^{14,15}, ephaptic (electrical field) effects^{4,16} and accumulation of K^+ in the extracellular space^{11,17,18}. Whether CA1 HPC are electrically coupled is still a matter of controversy^{19–21}, whereas evidence for the existence of ephaptic interactions has recently been reported⁴. Either of these mechanisms, however, is probably too fast to account simply for our findings. If activity spreads across the densely packed CA1 HPC population from cell to cell, each HPC body being about 20 μ m in diameter²², transmission across two adjacent HPC at a mean velocity of 1.7 $mm\ s^{-1}$ would require ~ 24 ms. In contrast, where electrical coupling has been demonstrated in the hippocampus by paired intracellular recordings, transmission of an action potential across two coupled neurones occurs within 1–2 ms^{14,15}. Ephaptic interactions among HPC also tend to synchronize their firing within a similarly brief period⁴. Therefore, cell-to-cell transmission of activity via these mechanisms would be expected to spread at a velocity of 20–40 $mm\ s^{-1}$, which is at least one order of magnitude higher than what we have observed. It is possible, however, that electrical coupling and ephaptic interactions influence the activated HPC

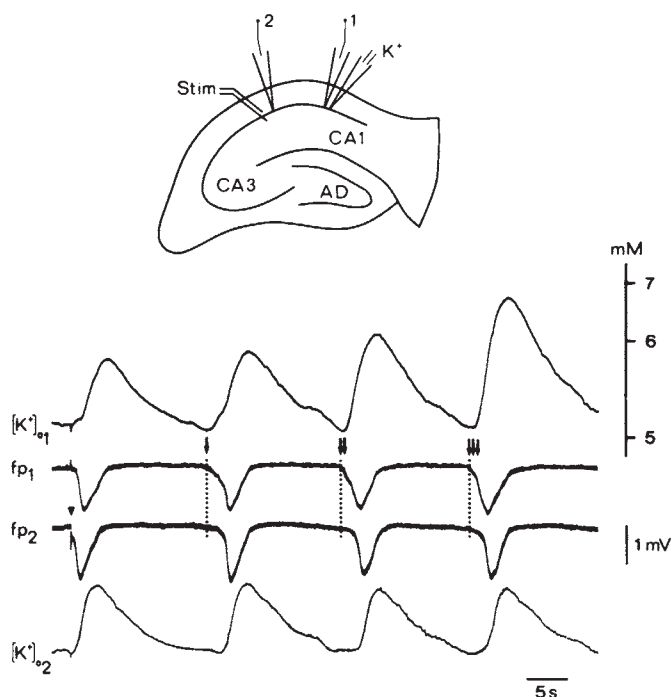


Fig. 3 Spreading NPS evoked at CA1 stratum pyramidale by direct electrical stimulation and K^+ pressure application, in a slice with blocked chemical synaptic transmission. Upper part illustrates experimental arrangement of stimulating electrode (Stim), recording K^+ -ISM (1 and 2) and K^+ pressure ejection micropipette (K^+), which were all positioned in the CA1 stratum pyramidale. ISM 1 and 2 were 500 μ m apart. The K^+ ejection micropipette, 50 μ m from ISM 1, contained 100 mM KCl and had a tip diameter of 1 μ m. KCl was applied by pressure pulses of 1 bar and 200 ms. Repetitive KCl pulses were delivered with an interpulse interval of 100 ms. The lower part demonstrates simultaneous recordings of the field potentials (fp_1 , fp_2) and the changes in $[K^+]_o$ ($[K^+]_{o1}$, $[K^+]_{o2}$) in response to a single-shock stimulation (marked by triangle) and to 1, 2 and 3 pulses of KCl (marked with arrows and dotted lines). Note the spread of the response from its site of initiation towards the distant ISM, and the similarity between the NPS evoked by electrical stimulation and by K^+ application. Similar ejections of NaCl (from a micropipette filled with 100 mM NaCl) were ineffective in eliciting a response.

to fire in synchrony, thereby giving rise to the frequently observed population spikes (Fig. 1B). These mechanisms have been suggested recently to account for the synchronization of CA1 HPC firing during short afterdischarges⁴ or during spontaneous field bursts³, which occur in solutions with low $[Ca^{2+}]$. Clearly, the fact that the NPS also spread in slices which did not exhibit synchronous population spikes, suggests that synchronization and spread are distinct phenomena, which may differ in their underlying mechanisms.

The observed slow spread of neural activity compares well with the time course of redistribution of $[K^+]_o$ transients through the glial 'spatial buffer' mechanism^{5,23,24}. Therefore, an alternative possible mechanism is that K^+ ions, released by active neurones, spread in the extracellular space^{13,18,23}, and depolarize to threshold neighbouring and perhaps remote quiescent neurones. This action would result in a relatively slow progressive spread of the 'discharge zone'. It has been shown in different preparations that the release of K^+ ions from active neurones can alter the excitability of neighbouring cells^{17,25,26}, and the fact that HPC CA1 neurones are tightly packed²⁷ may allow for an effective ' K^+ -coupling' mechanism. Indeed, in approximately 80% of our recordings, performed in 27 different slices, the $[K^+]_o$ increases associated with the spreading wave of activity, consisted of two distinct phases (Fig. 2). The first phase was slower and preceded the appearance of the NPS at the recording site; therefore, it may reflect the redistribution of K^+ from an adjacent active zone²⁴. The second phase consisted of

a much steeper and larger increase in $[K^+]_0$, and was correlated in time with the appearance of the NPS and the population spikes at the recording site. It is probably due to the local release of K^+ from the bursting neurones nearby. Thus, when the recording ISM was close to the stimulating electrodes, only a monophasic steep increase in $[K^+]_0$ was observed (Fig. 2).

To test the 'K⁺-coupling' hypothesis we examined whether similar spreading responses can be elicited by the local release of exogenous K^+ . In accordance with this hypothesis, we found that pressure ejection of KCl, but not NaCl, through the tip of a micropipette at the CA1 stratum pyramidale, could elicit typical NPS, similar to the responses evoked by direct electrical stimulation (Fig. 3). These responses also spread from their site of initiation through area CA1 (Fig. 3). Increases in the amount of locally applied K^+ hastened the appearance of the NPS, but did not significantly alter their amplitude (Fig. 3), indicating their all-or-none nature. Accordingly, reducing below a critical level the amount of applied K^+ , produced only a local response which did not develop into a full NPS, and did not spread to the remote recording ISM (not shown). As would be predicted from a 'K⁺-coupling' mechanism, we also found that elevating the $[K^+]$ in the perfusing solution by 1–2 mM, markedly accelerated the spread of the NPS, whereas lowering it by 1–2 mM had the opposite effect.

In sharp contrast to the responsiveness of CA1 HPC, we were consistently unable to evoke NPS and population spikes by focal stimulation at area CA3 in similar conditions. Furthermore, neural responses evoked at area CA1 always spread towards area CA3, but failed to invade it. A similar difference between the two hippocampal areas has been noted recently in their ability to generate spontaneous NPS in low- Ca^{2+} solutions^{3,11}. The reason for these differences is unknown, but it may be relevant that CA1 HPC are more densely packed than CA3 HPC²⁸, for the efficacy of both K⁺-coupling and ephaptic interactions should increase with neuronal density.

The question arises as to whether nonsynaptic transmission of neural activity could occur in the intact brain. At physiological $[Ca^{2+}]_0$, the spread of neural discharge through a cortical structure would be limited by powerful lateral and recurrent inhibitory synaptic mechanisms^{2,29}. However, intense cortical activity, observed most dramatically in the hippocampus and neocortex during an epileptic seizure, is accompanied by large decreases in $[Ca^{2+}]_0$ and increases in $[K^+]_0$ ^{8,9,13,30,31}. Indeed, in the baboon neocortex, reduction in $[Ca^{2+}]_0$ to levels below 0.1 mM during an ictal episode have now been found (S. Comtes, U.H., J. Louvel, C. Menini and R. Pumain, in preparation). Such ionic changes are expected to markedly reduce the efficacy of Ca^{2+} -dependent synaptic inhibition³¹ and augment HPC membranal excitability³², thereby facilitating nonsynaptic excitatory interactions among these neurones. The close contiguity of neuronal membranes in hippocampal area CA1²⁷ would make it a favourable substrate for such interactions, and may explain why seizure discharges in this structure do not seem to spread along anatomical paths by necessity⁶, and perhaps why the hippocampus is peculiarly susceptible to epilepsy³³.

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- Krnjevic, K. *Physiol. Rev.* **54**, 418–540 (1974).
- Shepherd, G. M. *The Synaptic Organization of the Brain* (Oxford University, New York, 1974).
- Jefferys, J. G. R. & Haas, H. L. *Nature* **300**, 448–450 (1982).
- Taylor, C. P. & Dudek, F. F. *Science* **218**, 810–812 (1982).
- Heinemann, U., Neuhaus, S. & Dietzel, I. in *Cerebral Blood Flow, Metabolism and Epilepsy* (eds Baldy-Moulinier, M. & Meldrum, B. S.) (John Libbey, London, in the press).
- Kreindler, A. *Prog. Brain Res.* **19**, 87–133 (1965).
- Prince, D. A. *Rev. Neurosci.* **1**, 395–415 (1978).
- Heinemann, U., Lux, H. D. & Gutnick, M. J. *Expl Brain Res.* **27**, 237–243 (1977).
- Heinemann, U., Konnerth, A., Louvel, G., Lux, H. D. & Pumain, R. in *Physiology and Pharmacology of Epileptogenic Phenomena* (eds Klee, M. R., Lux, H. D. & Speckmann, E. J.) 29–35 (Raven, New York, 1982).
- Skrede, K. K. & Westgaard, R. H. *Brain Res.* **35**, 589–593 (1971).

- Yaari, Y., Konnerth, A. & Heinemann, U. *Expl Brain Res.* **51**, 153–156 (1983).
- Konnerth, A. & Heinemann, U. *Brain Res.* **270**, 185–189 (1983).
- Heinemann, U. & Lux, H. D. *Brain Res.* **120**, 231–249 (1977).
- MacVicar, B. A. & Dudek, F. A. *Science* **213**, 782–785 (1981).
- MacVicar, B. A. & Dudek, F. A. *J. Neurophysiol.* **47**, 579–592 (1982).
- Green, J. D. & Petsche, H. *Electroenceph. clin. Neurophysiol.* **13**, 837–846 (1961).
- Yarom, Y., Grossman, Y., Gutnick, M. J. & Spira, M. F. *J. Neurophysiol.* **48**, 1089–1097 (1982).
- Hounsgaard, J. & Nicholson, C. *J. Physiol., Lond.* **340**, 359–388 (1983).
- Knowles, W. D., Funch, P. G. & Schwartzkroin, P. A. *Neuroscience* **7**, 1713–1722 (1982).
- Andrew, R. D., Taylor, C. P., Snow, R. W. & Dudek, F. E. *Brain Res. Bull.* **8**, 211–222 (1982).
- Taylor, C. P. & Dudek, F. E. *Brain Res.* **235**, 351–357 (1982).
- Lorenzo De No, R. *J. Psychol. Neurol.* **45**, 113–177 (1934).
- Dietzel, I., Heinemann, U., Hofmeier, G. & Lux, H. D. *Expl Brain Res.* **46**, 73–84 (1980).
- Gardner-Medwin, A. R. & Nicholson, C. *J. Physiol., Lond.* **335**, 375–392 (1983).
- Kocsis, J. D., Malenka, R. C. & Waxman, S. G. *J. Physiol., Lond.* **334**, 225–244 (1983).
- Katz, B. & Miledi, R. *Proc. R. Soc. B216*, 497–507 (1982).
- Green, J. D. & Maxwell, D. S. *Electroenceph. clin. Neurophysiol.* **13**, 837–846 (1961).
- Vinogradova, O. S. in *The Hippocampus Vol. 2* (eds Isaacson, R. L. & Pribram, K. H.) 3–69 (Plenum, London, 1975).
- Spencer, W. A. & Kandel, E. R. in *Basic Mechanisms of the Epilepsies* (eds Jasper, H. H., Ward, A. A. & Pope, A.) 575–603 (Little Brown, Boston, 1969).
- Dietzel, I., Heinemann, U., Hofmeier, G. & Lux, H. D. *Expl Brain Res.* **46**, 73–84 (1982).
- Krnjevic, K., Morris, M. E. & Reiffenstein, R. J. *Can. J. Physiol. Pharmac.* **58**, 579–583 (1980).
- Frankenhaeuser, B. & Hodgkin, A. L. *J. Physiol., Lond.* **137**, 218–244 (1957).
- Green, J. D. & Shimamoto, T. *Archs Neurol. Psychiat., Lond.* **70**, 687–702 (1953).

Are baclofen-sensitive GABA_B receptors present on primary afferent terminals of the spinal cord?

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The site of action of the antispasmodic drug baclofen has long been considered to reside in the spinal cord^{1,2} although supraspinal effects have also been reported^{3–6}. This β -chlorophenyl derivative of the neurotransmitter γ -aminobutyric acid (GABA) depresses both monosynaptic and polysynaptic transmission in the cord⁷ possibly through a decrease in transmitter release rather than by any antagonism at postsynaptic receptors^{7–10}. Recently, baclofen has been shown to be a selective ligand for a bicuculline-insensitive GABA receptor (GABA_B) site^{11,12} that occurs widely in the mammalian central nervous system including the spinal cord¹³. The apparent importance of the cord in the therapeutic effects of this drug prompted us to ask whether they involve GABA_B site activation. As an initial step we have located these receptors by autoradiography, comparing them with classical GABA_A sites. We report here that GABA_B sites, unlike GABA_A sites, are present in high concentrations in laminae I, II, III and IV of the dorsal horn and that after the neonatal administration of capsaicin this binding is reduced by 40–50%.

The distribution of GABA_A and GABA_B binding sites was determined in 10- μ m sections of rat spinal cord. Prepared sections were placed on glass slides and allowed to dry before storage at -20°C for periods up to 2 weeks. For the binding procedure the sections were thawed, washed and dried prior to incubation with ³H-GABA-containing solution at 20°C for 20 min. The incubation solution used for detection of GABA_A sites was Tris-HCl buffer solution (50 mM pH 7.4) containing 100 μM ($\pm$) baclofen. For GABA_B sites Tris-HCl buffer solution was used without baclofen but containing CaCl_2 (2.5 mM) and isoguvacine (40 μM) to suppress any binding to GABA_A sites^{13,14}. ³H-GABA (50 nM) was used as the receptor ligand

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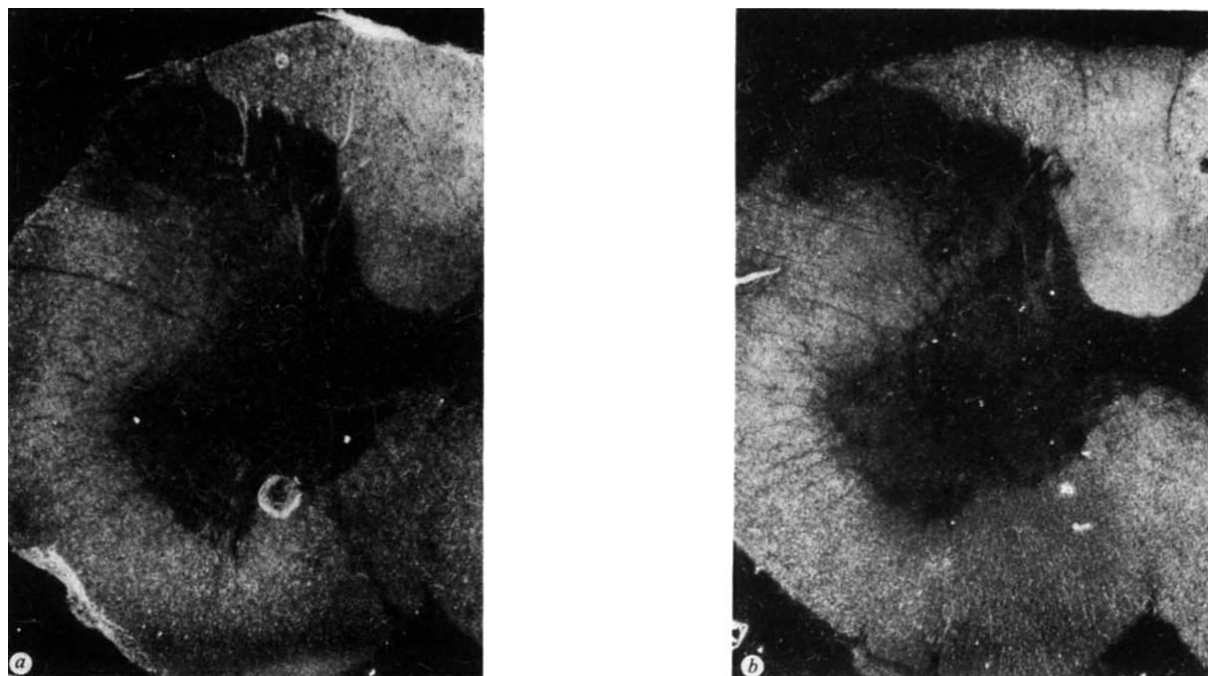


Fig. 1 Autoradiography of ^3H -GABA binding to GABA_A sites on rat spinal cord sections. The sections were prepared from a rat anaesthetized with pentobarbitone before intracardiac perfusion with 200 ml 0.1% paraformaldehyde in 0.01 M phosphate-buffered saline (pH 7.4). A segment of cervical cord was removed and immersed in isopentane in liquid nitrogen. Transverse sections ($10\ \mu\text{m}$) were mounted on glass slides and allowed to dry at ambient temperature for 1 h before storing at -20°C . For the binding procedure the sections were allowed to thaw and dry for 45 min before immersion in Tris-HCl solution (50 mM pH 7.4) containing sucrose (190 mM) for 60 min. After this the sections were dried before covering with $100\ \mu\text{l}$ of the Tris solution containing ^3H -GABA ($60\ \text{Ci mmol}^{-1}$, 50 nM) and ($\pm$)baclofen ($100\ \mu\text{M}$) without (*a*, total binding) or with (*b*, nonspecific binding) unlabelled isoguvacine ($100\ \mu\text{M}$). This was left in contact for 20 min after which the solution was aspirated off and the sections briefly rinsed twice in large volumes of Tris solution. The sections were allowed to dry and coverslips previously dipped in Ilford K5 emulsion (emulsion:water 1:1.5) were attached to the slides with Loctite glue and placed in a light-tight box containing silica-gel for 16 days at $+4^\circ\text{C}$. The emulsion was developed at 16°C in Kodak D19 developer (3 min) and fixed in 25% sodium thiosulphate solution. After several washes the slides were dried overnight and the coverslips fixed permanently in position with DPX mounting medium. Sections were viewed under dark-field optics with a Reichart Polywar microscope. Note in *a* the even density of grains throughout the grey matter indicating the presence of GABA_A sites in all laminae.

throughout these studies and was therefore present in both incubation solutions at the same concentration. After incubation, the sections were briefly rinsed twice before being air dried. The distribution of ^3H -GABA binding sites was then investigated using the dry-mounting autoradiographic technique previously described^{13,15}. Each slide was placed in close apposition to a coverslip coated with Ilford K5 photographic emulsion for 14–21 days. After developing, the grains per unit area were counted under dark-field optics. To facilitate comparison between the distributions of GABA_A and GABA_B sites, adjacent sections from the same cord were routinely incubated in appropriate conditions.

GABA_A -binding sites were evenly distributed throughout the grey matter of the spinal cord (Fig. 1), although there were

slightly more in the dorsal horn than in the ventral regions. In contrast, the distribution of GABA_B -binding sites was not uniform (Fig. 2). Although GABA_B sites could be detected throughout the grey matter, a clear band of high grain density was evident in the dorsal horn. This band was located mainly in laminae II and III (Fig. 2) but considerable numbers of grains were also evident in laminae I and IV and in the dorsal aspects of lamina X around the central canal.

A comparative analysis of the laminae grain densities indicated that there were more GABA_B sites in the dorsal laminae than in the ventral laminae whereas GABA_A sites showed very little fluctuation (Fig. 3).

Many studies of peripheral and central nervous tissue show that GABA_B sites can occur presynaptically^{11,16–21} although a

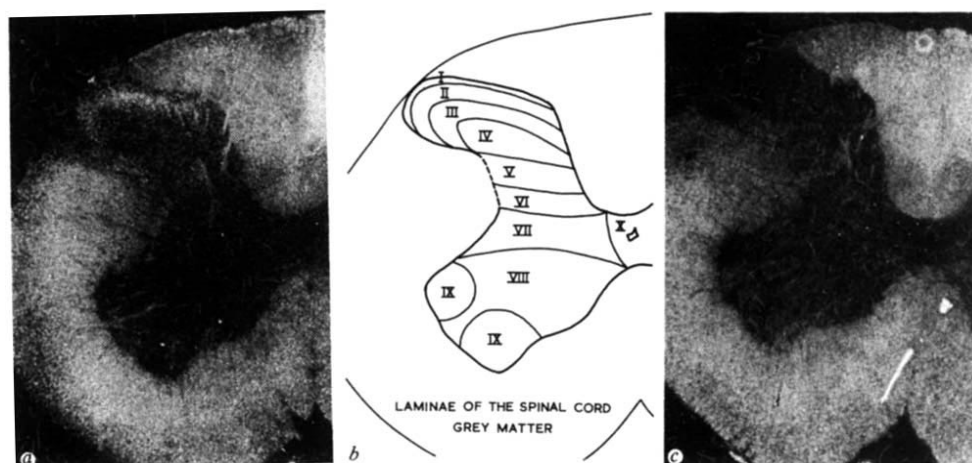


Fig. 2 Autoradiography of ^3H -GABA binding to GABA_B sites in rat spinal cord. Sections of spinal cord were prepared and treated as described for Fig. 1 except that the washing and incubating solutions contained 2.5 mM CaCl_2 and the incubating solutions contained $40\ \mu\text{M}$ isoguvacine. CaCl_2 facilitates binding to GABA_B sites while isoguvacine suppresses all binding to GABA_A sites¹². ($\pm$)Baclofen ($100\ \mu\text{M}$) was absent in *a* but present in *c* to obtain the background (non-specific) binding. *b*, Diagram of the separation between the laminae. In *a* grains corresponding to GABA_B sites appear right across the grey matter but the distribution is uneven. A dense band of grains is apparent in laminae II and III.

Table 1 Distribution of GABA_B sites in the dorsal horn of the rat spinal cord after neonatal administration of capsaicin

Lamina	Control (specific grains per 1,000 μm^2)	Capsaicin-treated (specific grains per 1,000 μm^2)	% Reduction
I	49.7 $\pm$ 5.4	29.8 $\pm$ 3.6	40.0
II _{outer}	60.2 $\pm$ 5.6	32.6 $\pm$ 3.1	45.8
II _{inner}	64.7 $\pm$ 6.2	32.8 $\pm$ 5.0	49.3
III	57.0 $\pm$ 8.6	26.8 $\pm$ 2.2	53.0
IV	40.7 $\pm$ 8.0	24.7 $\pm$ 2.6	39.3

Mean values $\pm$ s.e.m. Data obtained from average density in each lamina of six sections from capsaicin-treated animals and control littermates injected with vehicle alone.

postsynaptic location is possible²². The question arises therefore are GABA_B sites in the dorsal horn pre- or postsynaptic to the afferent fibre inputs? In all systems so far studied which show a presynaptic location, the activation of GABA_B sites reduces the evoked release of neurotransmitter. Such an action on terminals in the dorsal horn might well explain how baclofen acts as an analgesic²³⁻²⁵ and suppresses at least part of the symptoms of morphine withdrawal²⁶⁻²⁸.

Autoradiographic studies at the light microscope level in normal tissue cannot distinguish between pre- and postsynaptic locations. However, by selective elimination of the neural components, a distinction might become evident. It has been shown, for example, that after dorsal rhizotomy, opiate receptor binding decreases by 40% whilst leaving the binding of ³H-neurotensin intact²⁹. It is, therefore, unlikely that neurotensin binding sites are located presynaptically whereas there may be a population of opiate sites on the afferent terminal. Of course, this latter conclusion discounts any possible postsynaptic changes which may arise from presynaptic degeneration. Nevertheless, we have used this approach in an attempt to locate the GABA_B site. Selective degeneration of sensory afferent fibres was produced by the neonatal administration of capsaicin³⁰ following the procedure adopted by Faulkner and Growcott³¹. Three to four months after injection, transverse sections of cord were prepared. Sections were also prepared from littermates injected with vehicle alone. The binding of ³H-GABA was then performed as described above and the distribution of binding sites determined by autoradiography (Table 1). Capsaicin treatment reduced the number of GABA_B binding sites in substantia gelatinosa by 40–50% when compared with control littermates. Immunocytochemical analysis of the sections for the presence of substance P by the peroxidase–anti-peroxidase (PAP) technique³² indicated that the capsaicin treatment had produced a marked reduction in the peptide content of the gelatinosa when compared with littermates injected with vehicle alone. Similarly, a reduction in the fluoride-resistant acid phosphatase (FRAP) activity³³ was also noted. Both of these systems are considered to be markers for primary afferent terminals^{34,35}. Thus, taken together, these data confirm that at least some of the GABA_B sites detected in sections of normal spinal cord are present on primary afferent terminals. Whether the remaining sites are located presynaptically on descending pathways or on postsynaptic elements remains unclear.

The distribution of GABA_B sites in the cord corresponds closely with that of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD), a marker for GABA-containing synaptic terminals^{36,37}. The location of this enzyme in the dorsal horn agrees with a presynaptic function for GABA_B^{36,37}. Thus it is possible that the GABA released at these axo-axonic contacts is impinging on GABA_B receptors. Alternatively, as pointed out by Palacios *et al.*³⁸, bicuculline-sensitive GABA_A receptors may receive this innervation. These authors have described the location of ³H-muscimol-binding sites in spinal cord incubated in conditions which would facilitate binding to GABA_A sites, but only a low concentration of sites could be discerned. Singer and Placheta³⁹, using membrane pellets prepared from rat dorsal horn, have shown that neonatal capsaicin treatment can reduce the number of ³H-muscimol-binding sites

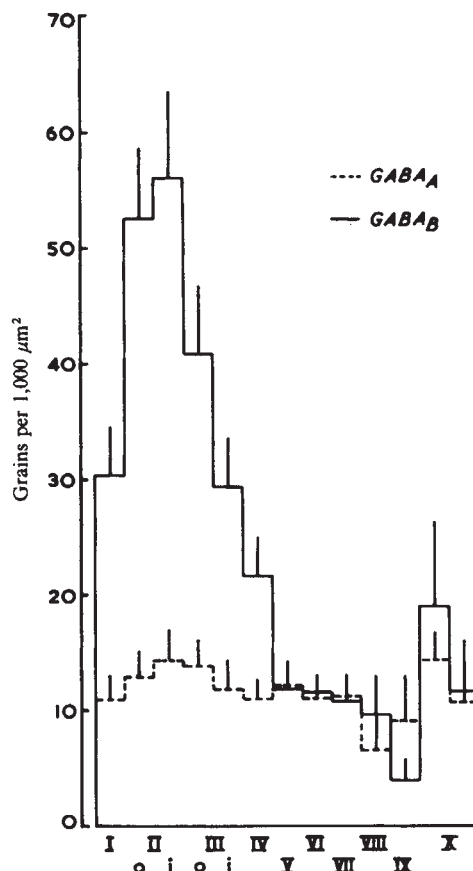


Fig. 3 Analysis of the distributions of GABA_A and GABA_B sites in rat spinal cord sections. Transverse sections of spinal cord were prepared and incubated with ³H-GABA for GABA_A or GABA_B site detection as described in the legends to Figs 1 and 2. Autoradiograms were subsequently prepared as described in Fig. 1. Grain densities in the individual laminae were determined by counting three areas in each lamina or lamina region (inner, i; or outer, o) from the spinal cords of six rats. Measurements were made after ascertaining that grain accumulation was linear with time. Mean values $\pm$ s.e.m. (vertical lines) are shown. The solid outline represents GABA_B site concentrations whereas the broken outline represents GABA_A site concentrations.

by 20–30%. This would support the long-established view that bicuculline-sensitive GABA receptors (GABA_A sites) are present on primary afferent terminals. However, since the concentration of GABA_B sites is much higher they may have a greater role in GABA-mediated axo-axonic transmission in the spinal cord.

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- Bein, H. J. in *Spasticity—a topical survey* (ed. Birkmayer, W.) (Hans Huber, Vienna, 1972).
- Fehr, H. U. & Bein, H. J. *J. int. Med. Res.* **2**, 36 (1974).
- Saelens, J. K., Welch, J., Robson, R. D. & Roffman, M. *Fedn Proc.* **36**, 395 (1977).
- Davies, J. & Watkins, J. C. *Brain Res.* **70**, 501–505 (1974).
- Olpe, H. R., Koella, W. P., Wolf, P. & Haas, H. L. *Brain Res.* **134**, 577–580 (1977).
- Lanthorn, T. H. & Cotman, C. W. *Brain Res.* **225**, 171–178 (1981).
- Davies, J. *Br. J. Pharmac.* **72**, 373–384 (1981).
- Fox, S., Krnjevic, K., Morris, M. E., Puil, E. & Werman, R. *Neuroscience* **3**, 495–515 (1978).
- Davidoff, R. A. & Sears, E. S. *Neurology* **24**, 957–963 (1974).
- Henry, J. L. *Neuropharmacology* **21**, 1085–1093 (1982).
- Bowery, N. G. *et al. Eur. J. Pharmac.* **71**, 53–70 (1981).
- Hill, D. R. & Bowery, N. G. *Nature* **290**, 149–152 (1981).
- Wilkin, G. P., Hudson, A. L., Hill, D. R. & Bowery, N. G. *Nature* **294**, 584–587 (1981).
- Bowery, N. G., Hill, D. R. & Hudson, A. L. *Br. J. Pharmac.* **78**, 191–206 (1983).
- Young, W. S. & Kuhar, M. J. *Brain Res.* **179**, 255–270 (1979).
- Bowery, N. G. *et al. Nature* **283**, 92–94 (1980).
- Muhyaddin, M., Roberts, P. J. & Woodruff, G. N. *Br. J. Pharmac.* **77**, 163–168 (1982).

18. Hughes, P. R., Morgan, P. F. & Stone, T. W. *Br. J. Pharmac.* **77**, 691–695 (1982).
19. Cain, C. R. & Simmonds, M. A. *Neuropharmacology* **21**, 371–373 (1982).
20. Kilpatrick, G. J., Muhyaddin, M. S., Roberts, P. J. & Woodruff, G. N. *Br. J. Pharmac.* **78**, Suppl. 6P (1983).
21. Fillenz, M. & Fung, S. C. *J. Physiol.*, **339**, 390 (1983).
22. Bowerly, N. G., Price, G. W., Turnbull, M. J. & Wilkin, G. P. *Br. J. Pharmac.* **79**, Suppl. 189P (1983).
23. Cutting, D. A. & Jordan, C. C. *Br. J. Pharmac.* **54**, 171–179 (1975).
24. Levy, R. A. & Proudfoot, H. K. *J. Pharm. exp. Ther.* **202**, 437–445 (1977).
25. Cutting, D. A. & Jordan, C. C. *Scot. med. J.* **25**, S17–S22 (1980).
26. Brodie, M. E. & McQueen, E. G. *Proc. Univ. Otago med. Sch.* **53**, 40–41 (1975).
27. Ladewig, D. *Neurol. Psychiat. Prax. (D)* **10** (1980).
28. Bowerly, N. G. *Trends pharmac. Sci.* **3**, 400–403 (1982).
29. Ninkovic, M., Hunt, S. P. & Kelly, J. S. *Brain Res.* **230**, 111–119 (1981).
30. Jancso, G., Kiraly, E. & Jancso-Gabor, A. *Nature* **270**, 741–743 (1977).
31. Faulkner, D. C. & Growcott, J. W. *J. pharm. Pharmac.* **32**, 656–657 (1980).
32. Sternberger, L. A. in *Immunocytochemistry*, 104–169 (Wiley, New York, 1979).
33. Disbrey, B. D. & Rack, J. H. *Histological Laboratory Methods* (Livingstone, Edinburgh, 1970).
34. Gamse, R., Holzer, P. & Lembeck, F. *Br. J. Pharmac.* **68**, 207–213 (1980).
35. Nagy, J. I., Hunt, S. P., Iversen, L. L. & Emson, P. C. *Neuroscience* **6**, 1923–1924 (1981).
36. McLaughlin, B. J., Barber, R., Saito, K., Roberts, E. & Wu, J.-Y. *J. comp. Neurol.* **164**, 305–322 (1975).
37. Barber, R. P., Vaughn, J. E., Saito, K., McLaughlin, B. J. & Roberts, E. *Brain Res.* **141**, 35–55 (1978).
38. Palacios, J. M., Wamsley, J. K. & Kuhar, M. J. *Brain Res.* **222**, 285–307 (1981).
39. Singer, E. & Placheta, P. *Brain Res.* **202**, 484–487 (1980).

Bonding of molecular oxygen to T state human haemoglobin

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Haemoglobin (Hb) is the tetrameric protein molecule that in vertebrate blood transports oxygen from the lungs to the tissues. This function depends on four subunits in the molecule binding cooperatively so that their affinity for oxygen increases as the level of oxygenation increases. X-ray analysis has shown that deoxyhaemoglobin, which has a low oxygen affinity, and oxyhaemoglobin, which has a high oxygen affinity, differ principally in their subunit or quaternary structures¹, referred to as the T and R states, respectively. As it switches from the T state to the R state during oxygenation, Hb increases its oxygen affinity. However, the structural pathway between deoxy- and oxyhaemoglobin is not known, principally because there has been no accurate structural knowledge of the intermediate states. We report here the crystal structure of T state human Hb in which the α chains are oxygenated and the β subunits are oxygen-free. In this crystal the Hb appears to be in an intermediate state between the unliganded T state and the liganded R state. There is also evidence that the Hb molecule operates by loading and unloading the β haems and thus the α -oxy, β -deoxy Hb crystal may represent a physiologically important state².

Perutz has proposed³ that oxygen's binding to Fe(II) in deoxyhaemoglobin alters the stereochemistry at the haem which destabilizes the T state quaternary structure, favouring its switch to the R state quaternary organization with its higher oxygen affinity. The crystal structures of deoxyhaemoglobin⁴ and oxyhaemoglobin⁵ have been determined with considerable accuracy and consequently there is a detailed atomic description for these two structures⁶. The partially oxygenated T-state Hb crystal has parallels with the crystalline intermediate produced by Anderson⁷ and agrees with the α subunits in the T structure having the higher oxygen affinity because the binding of oxygen to the β haems is hindered by (CH₃)₇ of the distal valine^{3,8}. The crystals were grown from polyethylene glycol (PEG), following the procedure of Ward and his colleagues⁹, except that air was allowed entry into the crystallization vessels and the crystals were transferred to more concentrated solutions of PEG before

Table 1 Stereochemical features of the haem in various molecules

Molecule	Fe–O (Å)	Fe– $\hat{\text{O}}$ –O (°)	O–O (Å)	Fe–N ϵ (His F8) (Å)	Fe–Ct (Å)
T state Hb(PEG)					
α_1 -oxy	1.81	160 $\pm$ 5	1.23	2.23†	2.10§ 0.2*
α_2 -oxy	1.81	160 $\pm$ 5	1.24	2.50†	2.39§ 0.2*
β_1 -deoxy	—	—		2.21†	2.39§ 0.3*
β_2 -deoxy	—	—		2.20†	2.14§ 0.3*
T state Hb ⁵					
α -deoxy				2.00‡	0.60
β -deoxy					
R state Hb ⁴					
α -oxy	1.67	153	1.22	1.94	1.94 0.13
β -oxy	1.83	158	1.24	2.06	2.06 0.08
Mb ¹⁴					
deoxy	—	—	—	2.22	2.22 0.42
oxy	1.83	115	1.18	2.07	2.07 0.18

Fe–Ct, the distance from the haem plane to the Fe(II).

* The haem plane is defined by the least squares plane through the four pyrrole nitrogens.

† These values (e.s.d. 0.1–0.2 Å) were obtained after six cycles of refinement with Fe–N ϵ restraints removed. The Fe–N ϵ distance in the initial set of coordinates lay between 2.08–2.11 Å, essentially the distance to which it had been restrained.

‡ Diamond refinement at 2.5 Å (ref. 5).

§ Refinement values obtained with the Jack–Levitt procedure¹⁵; weak restraints were applied to the Fe–N ϵ distance.

X-ray study¹⁰. A number of crystal forms were produced with these conditions which were identifiable from their X-ray diffraction patterns¹¹. They are all similar to the crystal form reported by Ward *et al.*⁹, containing one complete molecule in the asymmetric unit. Preliminary studies at 3.5 Å resolution on two of the more reproducible forms showed that one was apparently unliganded while the other appeared to contain liganded α haems (unpublished work).

A 2.1 Å dataset was subsequently collected at Laboratoire pour l'Utilisation de Rayonnements Electromagnetique (LURE) on a partially liganded crystal. The crystal structure was refined from the human deoxyhaemoglobin coordinates⁶, positioned correctly in the PEG Hb cell by molecular replacement calculations¹¹. The initial agreement factor $R(=\sum|F_o|-|F_c|/\sum|F_o|)$ was 0.52; on refinement, this has been reduced to 0.21.

The refinement of the crystal structure was carried out using the fast Fourier least squares procedure of Agarwal¹². At a resolution of 2.1 Å the calculated shifts are inaccurate and the correct peptide geometry was imposed¹³ on the atomic coordinates after each shift calculation.

Refinement of the well-ordered atoms is essentially complete with the estimated error in their atomic positions being between 0.1 and 0.2 Å. The further lengthy crystallographic refinement needed on some of the poorly ordered residues, and in improving and extending the descriptions of the solvent structure, will be published elsewhere. It will not alter materially the picture we have of the stereochemical features at the haem groups presented here.

The bond distances and angles at the Fe(II) which are most liable to be affected by ligation unfortunately presented a particular difficulty since they are not known with the same accuracy as those in peptides. The effect of different restraints of geometries on the refinement of the groups about the haem was explored. A parallel refinement was also carried out with the Jack–Levitt procedure which was used in the oxyhaemoglobin study¹⁵. The coordinate sets finally arrived at agreed satisfactorily within their standard deviations, the well-defined atoms in the haems differing by less than 0.18 Å. There is nonetheless evidence from the refinement calculations and from the electron density (Figs 1 and 2 and Table 1) that the Fe–N ϵ (His F8) distance in the α_2 subunit is rather longer than the normal values, which raises the possibility that the Fe is in the high-spin state.

The electron density at the α haems and the β haems is illustrated in Figs 1 and 2. The oxygen peak is well defined and

Fig. 1 Stereo view of the electron density at and surrounding the α haems calculated with $2F_o - F_c$ coefficients and excluding the haem and proximal and distal histidines from the phasing. The atoms in the haems and proximal and distal histidines are shown: *a*, $\alpha 1$; *b*, $\alpha 2$.

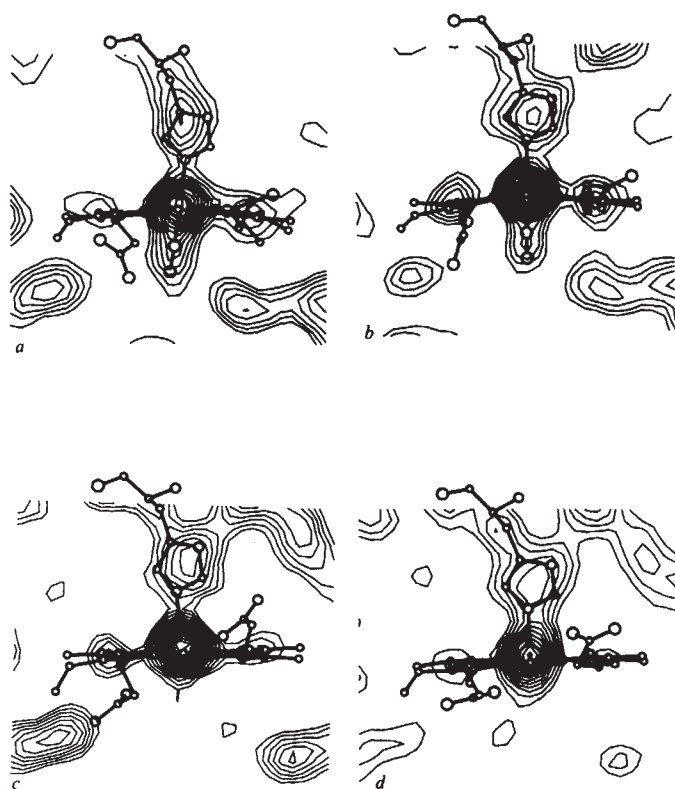
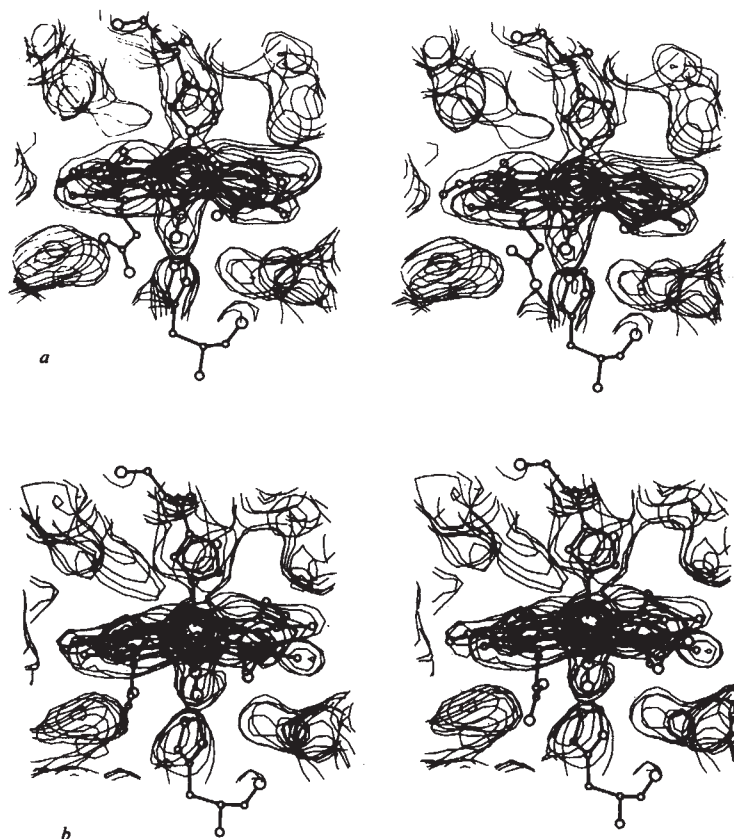


Fig. 2 The haem and proximal histidine viewed edge-on. A section of the electron density, calculated as for Fig. 1, through the Fe and proximal histidine (and oxygen in the case of the α haems) is shown: *a*, $\alpha 1$; *b*, $\alpha 2$; *c*, $\beta 1$; *d*, $\beta 2$.

its height suggests that it is fully occupied in the crystal. Calculations on the oxygen's occupancy confirm this.

Two notable features in the partially oxygenated molecule are, first, that the arrival of the oxygens at the α haems has produced only limited movement from the T state. Second, there is a marked folding of the haems in both α subunits. (Refinement of high salt deoxyhaemoglobin at 1.7 Å (G. Fermi, personal communication) and PEG deoxyhaemoglobin at 2.1 Å (unpublished results) reveals that the α haems in these molecules are buckled in an identical fashion to that observed in the partially oxygenated form.) This stereochemistry lifts the Fe(II) about 0.2 Å out of the plane defined by the four pyrrole nitrogens; note that the Fe(II) is at, or very near to, the apex of the pyramid formed by the planes of the four pyrrole rings. In contrast, the β haems are essentially flat and the 5 coordinate Fe(II) is about 0.3 Å out of the plane (Fig. 2).

This indicates that the globin is restraining the haem and the oxygen from assuming a more favourable stereochemistry, thereby reducing the stability of the T state relative to the R state. The particularly close interaction between the haemoglobin molecules in these crystals undoubtedly serves to stabilize the T state quaternary structure and is evidently preventing the switch to the R state and inhibiting further ligation of the β haems.

Detailed analysis of the structural changes associated with oxygen's binding can only be made when the fully deoxygenated molecule's crystal structure is refined and can be compared to the liganded molecule. Then the significance of the Fe-N ϵ distances and of the different oxygen interactions in the two α subunits can be better assessed. Comparison is being extended to the structures of the deoxy Hb and the fully liganded T state Hb aquomet and azide species. In this way we hope to elucidate how the steric effects in the Fe coordination and in the haem's geometry lead to the structural movements in the globin that destabilize the T state, thus favouring the R state and generating increased oxygen affinity.

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1. Baldwin, J. & Chothia, C. *J. molec. Biol.* **129**, 175–220 (1979).
2. Weber, G. *Nature* **300**, 603–606 (1982).
3. Perutz, M. F. *Nature* **237**, 495–499 (1977).
4. Fermi, G. *J. molec. Biol.* **97**, 237–256 (1975).
5. Shaanan, B. *Nature* **296**, 683–684 (1982).
6. Fermi, G. & Perutz, M. F., in *Haemoglobin and Myoglobin* (eds Phillips, D. C. & Richards, F. M.) (Clarendon, Oxford, 1981).
7. Anderson, L. *J. molec. Biol.* **94**, 33–49 (1975).
8. Viggiano, G. & Ho, C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3673–3677 (1979).
9. Ward, K. B., Wishner, B. C., Lattman, E. E. & Love, W. E. *J. molec. Biol.* **98**, 161–171 (1975).
10. Grabowski, M. J. *et al. Biochem. J.* **171**, 277–279 (1978).
11. Derewenda, Z. S., Dodson, E. J., Dodson, G. G. & Brzozowski, A. M., *Acta crystallogr.* **A37**, 407–413 (1981).
12. Agarwal, R. C., *Acta crystallogr.* **A34**, 791–809 (1978).
13. Dodson, E. J., Isaacs, N. W. & Rollett, J. R. S., *Acta crystallogr.* **A34**, 931–935 (1978).
14. Phillips, S. E. V. *J. molec. Biol.* **142**, 531–554 (1980).
15. Jack, A. & Levitt, M. *Acta crystallogr.* **A34**, 931–935 (1978).

Ferrara β^0 thalassaemia caused by the β^{39} nonsense mutation

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Ferrara type of β^0 thalassaemia has two unusual features: first, normal β -globin chain synthesis is inducible either in cell-free systems prepared from patients' reticulocytes by adding supernatant factors from non-thalassaemic reticulocyte lysates¹ or in heterologous cell-free translation of thalassaemic mRNA²; second, β -globin synthesis is inducible in patients *in vivo* after blood transfusion^{3,4}. We now describe a molecular lesion of the β -globin gene that is common to nine cases of Ferrara β^0 thalassaemia but cannot be reconciled with the inducible response.

DNA from nine patients with this disorder was analysed by the synthetic deoxyoligonucleotide method^{5,6}. Two nonadecamers were used as hybridization probes, one homologous to the normal β -globin gene sequences at the region corresponding to β^{39} , and the other to the same region of a β^0 -thalassaemia gene caused by a β^{39} (Gln, CAG $\rightarrow$ TAG) nonsense mutation⁷. In well-defined hybridization and washing conditions, each probe hybridizes only to its homologous sequence. The DNA sequences from 17 chromosomes of the nine patients hybridized with the β^0 -thalassaemia probe (Fig. 1), indicating that the primary lesion responsible for Ferrara β^0 thalassaemia is the β^{39} nonsense mutation. The nature of the mutation in the remaining chromosome has not been defined. The β^{39} nonsense mutation is widespread throughout the Mediterranean region. It has been detected in other parts of Italy, Greece, Turkey, Morocco, Lebanon and Spain (M.P. and Y.W.K., unpublished data).

We cannot reconcile our present findings with the previously reported results. One possible explanation for the *in vitro* induction of β -globin synthesis is that suppressor tRNA was present

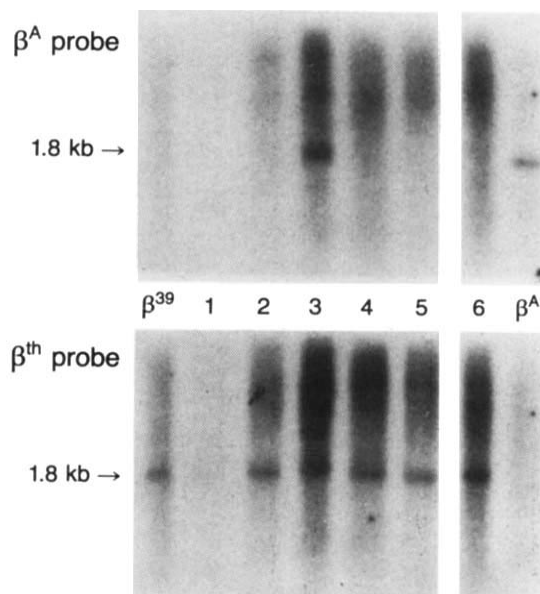


Fig. 1 Autoradiographs of gels containing DNA from Ferrara patients, hybridized with synthetic deoxyoligonucleotide probes. The method used has been described in detail⁷. Briefly, DNA was digested with *Bam*HI, electrophoresed on agarose gels, and hybridized with two ³²P-labelled nonadecamer probes. The β^A probe has the sequence 5' CCTTGGACCCAGAGGTTCT 3' and is homologous to the coding strand of the normal β-globin gene at the position corresponding to amino acid numbers 35–42. The βth probe has the sequence 5' AGAACCTCTAGGTCCAAGG 3' and is homologous to the noncoding strand of the β-thalassaemia gene with the β³⁹ (Gln, CAG $\rightarrow$ TAG) nonsense mutation at the same amino acid position. Results of samples obtained from six of the nine Ferrara β⁰ thalassaemia patients (1–6) are compared with DNA from a Sardinian with the same β³⁹ nonsense mutation (β³⁹) and with DNA from a non-thalassaemic individual (β^A). Patient 3 is the only double heterozygote for the β³⁹ nonsense mutation and another undefined β-thalassaemia lesion. All other patients were homozygous for the β³⁹ nonsense mutation.

in the supernatant factors used. It has been demonstrated previously that the amber termination codon in β^0 thalassaemia can be suppressed with an amber suppressor tRNA^{8–10}. However, although a tRNA with a UGA suppressor has been described in rabbit reticulocytes¹¹, no amber suppressor has yet been detected in humans. Furthermore, the suppressor tRNA theory cannot explain the *in vivo* transfusion results. We have examined transfusion records of 100 Sardinian β⁰ thalassaemia patients with the same β³⁹ nonsense mutation and did not observe induction of β-globin synthesis at any time following transfusion. The Ferrara patients should be reinvestigated in light of the present findings.

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Received 1 September; accepted 18 October 1983.

1. Conconi, F., Rowley, P. T., del Senno, L., Pontremoli, S. & Volpato, S. *Nature new Biol.* **238**, 83–87 (1972).
2. Conconi, F. *et al. Ann. N.Y. Acad. Sci.* **334**, 120–131 (1980).
3. Conconi, F. *et al. Nature* **254**, 256–259 (1975).
4. Ottolenghi, S. *et al. Nature* **266**, 231–234 (1977).
5. Wallace, R. B., Schold, M., Johnson, M. J., Dembek, P. & Itakura, K. *Nucleic Acids Res.* **9**, 3647–3656 (1981).
6. Conner, B. J., Reyes, A. A., Morin, C., Itakura, K. & Wallace, R. B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 278–282 (1983).
7. Pirastu, M. *et al. New Engl. J. Med.* **309**, 284–287 (1983).
8. Chang, J. C., Temple, G. F., Trecartin, R. F. & Kan, Y. W. *Nature* **281**, 602–603 (1979).
9. Trecartin, R. F. *et al. J. clin. Invest.* **68**, 1012–1017 (1981).
10. Temple, G. F., Dozy, A. M., Roy, K. L. & Kan, Y. W. *Nature* **296**, 537–540 (1982).
11. Geller, A. I. & Rich, A. *Nature* **283**, 41–46 (1980).

For more rigorous quantitative analysis, total RNA from spleen or peripheral blood lymphocytes was hybridized in solution to saturation with an excess of a single-stranded, uniformly ^{32}P -labelled restriction fragment containing $V_{\kappa}1$, $V_{\kappa}3$ or C_{κ} sequences. RNA-DNA hybrids were detected by resistance to the single-strand-specific exonuclease VII (exoVII). An exonuclease was chosen to preserve hybrids with internal mismatches. The endonuclease S_1 reduced the yield of full-length resistant hybrids with the V_{κ} probes by fourfold relative to exoVII. The restriction fragment probes had irrelevant vector or intron sequences at the ends, so the shorter nuclease-resistant RNA-DNA hybrids could be resolved on a gel. Figure 3 shows an example of the results. Arrows indicate the exoVII-resistant fragments corresponding to full-length protection of the immunoglobulin-coding sequences. The results of several experiments with RNA from two spleens are summarized in Table 1. The data were quantitated by counting the radioactivity in bands excised from gels. After correcting for the different numbers of ^{32}P -labelled nucleotides in the nuclease-resistant fragments, the relative amounts of hybridization of the V_{κ} and C_{κ} probes was calculated. In both spleens over 20% of κ mRNA was detected by each of the two V_{κ} probes.

It was important to establish whether or not the $V_{\kappa}1$ and $V_{\kappa}3$ genes cross-hybridize with the same mRNAs in the nuclease-resistance assay. In a control experiment, the *HK101* $V_{\kappa}1$ sequence did not form an exoVII-resistant hybrid with $V_{\kappa}3$ mRNA from the NG9 hybridoma (data not shown), showing that these two V_{κ} probes cannot cross-react with identical subsets of the κ mRNA population. When a mixture of the two probes ($V1+3$) was hybridized to RNA in a 5:1 DNA:RNA molar ratio of each, the amount of hybridization equalled the sum of that observed with each probe alone in a 10:1 molar ratio ($V1+V3$; see Fig. 3, Table 1). This result shows that the hybridizations proceeded to completion and that the two cloned V_{κ} genes react with completely non-overlapping subsets of κ mRNA. These experiments contrast with filter hybridizations in which $V_{\kappa}1$ and $V_{\kappa}3$ sequences readily cross-react. The reasons for the greater specificity of the RNA-DNA solution hybridization experiments are that the hybrids are formed in more stringent conditions and then subjected to nuclease digestion. It is most likely the $V_{\kappa}1$ and $V_{\kappa}3$ genes only form exoVII-resistant hybrids with mRNAs from their respective subgroups.

The family of germ-line genes in Fig. 2a includes subgroups 1 and 3 sequences and their expression is therefore given by the sum of the $V_{\kappa}1$ and $V_{\kappa}3$ components of spleen mRNA. In spleen *a*, at least 61% and in spleen *b*, at least 53% of κ mRNA is homologous to these 25 germ-line *V* genes (Table 1). These are minimum values because it is unlikely that the two V_{κ} probes would form full-length exoVII-resistant hybrids with every transcript homologous to this family of germ-line genes. The exoVII protection experiments therefore substantiate the value of 75% determined by Northern blot hybridization (Fig. 2b). The large amount of $V_{\kappa}1$ and $V_{\kappa}3$ mRNA in spleen was also observed in peripheral blood lymphocytes (PBLs) from another donor. Over

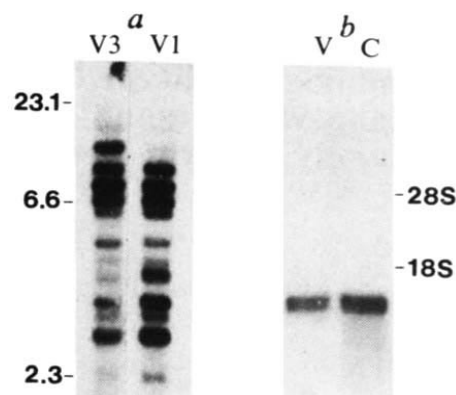


Fig. 2 *a*, Southern blot hybridization of $V_{\kappa}1$ and $V_{\kappa}3$ probes to human DNA. The author's peripheral blood DNA (10 μg) digested with *Bgl*II was electrophoresed in a 1% agarose gel and transferred to nitrocellulose. Adjacent tracks from the gel were hybridized to $V_{\kappa}1$ - and $V_{\kappa}3$ -specific restriction fragments labelled by nick translation or filling-in after exonuclease digestion with T4 DNA polymerase. The probes were the 558-bp *Pst*I fragment from *HK101/80*³ which extends from 130 bases upstream of the gene to codon 79 and the 266-bp insert from *NG9/3* excised with *Bam*HI. Hybridization was according to ref. 23 in 5 $\times$ SSC, 50% formamide at 42 $^{\circ}\text{C}$ for 24 h. The blots were washed at 65 $^{\circ}\text{C}$ in 2 $\times$ SSC, 0.1% SDS for 30 min and then in 50 mM Tris-HCl, pH 7.6, 10 mM sodium pyrophosphate, 2 mM Na_2EDTA , 1 $\times$ Denhardt's solution, 0.05% SDS, 0.05% sarkosyl for 15 min. *b*, Northern blot hybridization of V_{κ} and C_{κ} probes to human spleen RNA. Poly(A)⁺ RNA (2 μg) from spleen *b* was electrophoresed in a 1.2% formaldehyde agarose gel and blotted onto nitrocellulose²⁴. Adjacent tracks were hybridized to nick-translated restriction fragments (about 1×10^8 d.p.m. per μg , 1×10^6 d.p.m. ml^{-1}) in conditions described in Fig. 2a. The V_{κ} probe was the same fragment of the *HK101* gene used in *a*. The C_{κ} probe was the 469-bp insert from the clone M13/*C_{\kappa}6* excised with *Bam*HI¹⁰. The fragment extends downstream from codon 115 in the constant region. The positions of *Xenopus laevis* ribosomal RNA markers are shown.

60% of κ mRNA from PBLs hybridized to a mixture of $V_{\kappa}1$ and $V_{\kappa}3$ probes (Fig. 3). The diversity of the V_{κ} mRNA sequences detected is not known, although this important question could be answered by sequencing lymphocyte cDNA clones. What is clear from these experiments is that the majority of κ mRNA in human lymphocytes is encoded by about 25 germ-line *V* genes.

The frequencies of $V_{\kappa}1$ and $V_{\kappa}3$ mRNAs in spleen correspond with the frequencies of these subgroups in serum κ chains of various individuals and in the panel of sequenced myeloma proteins. The amount of subgroup 3 among serum κ -chains has been measured by serological means¹², by N-terminal sequencing of pooled light chains¹³ and by isolation of a subgroup-specific peptide¹⁴. These studies gave average values of 21–31%. Thirty-four per cent (62/182) of sequenced myeloma chains

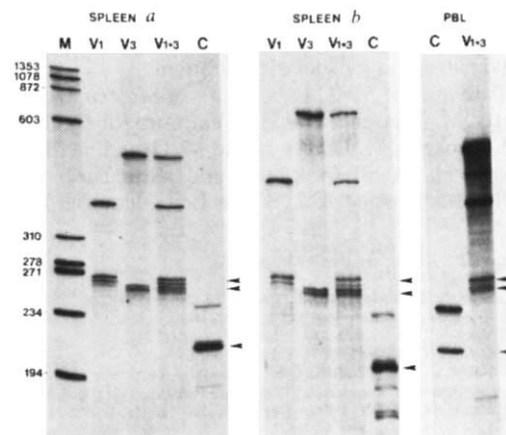
Table 1 Amounts of hybridization of V_{κ} and C_{κ} probes to spleen RNA

	<i>C</i>	<i>V1</i>	<i>V1/C</i>	<i>V3</i>	<i>V3/C</i>	<i>V1+V3/C</i>	<i>V1+V3/V1+3</i>
Spleen <i>a</i>	2.54 $\pm$ 0.14 (3)	1.00 $\pm$ 0.20 (6)	39%	0.55 $\pm$ 0.03 (5)	22%	61%	100% $\pm$ 14% (5)
Spleen <i>b</i>	0.81 $\pm$ 0.03 (3)	0.19 $\pm$ 0.04 (3)	23%	0.24 $\pm$ 0.03 (3)	30%	53%	101% $\pm$ 27% (3)

Values are given in pmol $\times 10^{-4}$ of probe hybridized per μg of total RNA. Means and standard deviations from at least three independent experiments are given. The number of determinations is given in parentheses. Gel slices were counted with 63% efficiency in 0.2 ml solute (Packard) plus 2 ml Omnifluor (NEN). Actual values were between 200 and 1,200 c.p.m. Background from an equal area of gel immediately above the bands was subtracted. Background values were $\leq 35\%$ of total counts. The amount of probe hybridized was calculated from the number of T residues in each exoVII-resistant fragment, and the specific activity of [α - ^{32}P]dTTP in the probe synthesis reaction (see Fig. 3, Methods). The number of Ts in the *V1*, *V3* and *C* fragments were 60, 52 and 37, respectively. The RNA preparation from spleen *b* was degraded relative to that from spleen *a* and this explains the lower amounts of hybridization observed. $V1+V3/V1+3$ is the ratio of the sum of the amounts of hybridization of the $V_{\kappa}1$ and $V_{\kappa}3$ probes alone (0.008 pmol of each per hybridization) to the amount of hybridization of a mixture of the $V_{\kappa}1$ and $V_{\kappa}3$ probes (0.004 pmol of each per hybridization).

Fig. 3 Analysis of spleen and peripheral blood lymphocyte (PBL) RNA with $V_{\kappa}1$, $V_{\kappa}3$ and C_{κ} probes by *exoVII* protection. For each track total RNA (spleen, 3 μ g; PBLs, 1 μ g) prepared according to ref. 27 was hybridized to a single-stranded uniformly labelled probe, the hybrids digested with *exoVII* and electrophoresed on a 5% sequencing gel. Two spleens, *a* and *b*, were obtained from transplant donors. PBLs from a third individual were purified by density gradient sedimentation on Lymphoprep (Pharmacia). The tracks are labelled with the relevant probes V_1 , V_3 , C or $V_1 + V_3$ which was an equimolar mixture of the two V probes. The fragments corresponding to full-length protection of immunoglobulin sequences in the probe are arrowed. Some undigested probe is evident in each track. The larger number of less than full-length resistant fragments seen in spleen *b* is due to partial degradation of this RNA sample as judged from ethidium bromide-stained agarose gels. DNA size markers are indicated for spleen *a*. The three RNA samples shown were analysed on separate gels.

Methods: Preparation of single-stranded probes. The 'prime-cut' probes complementary to mRNA were made by primed synthesis on a single-stranded M13 template followed by cutting with a restriction enzyme. The fragment extending from the primer to the restriction site was isolated from the longer M13 template strand on a sequencing gel. In a typical reaction 1 μ g of template (0.4 pmol) was annealed to 5 ng of universal 17mer sequencing primer²⁵ in 5 μ l by heating for 30 s to 80 °C and incubating at room temperature for 20 min in 20 mM Tris-HCl pH 8.5, 100 mM NaCl, 20 mM MgCl₂, 2 mM dithiothreitol²². The annealing mix was diluted twofold by addition of three unlabelled nucleotide triphosphates to 100 μ M and one [α -³²P]-labelled triphosphate at 52.5 μ M with a specific activity of 37 Ci mmol⁻¹. Klenow fragment was added to 250 U ml⁻¹ and the reaction incubated at room temperature for 15 min. The reaction was terminated by heating to 65 °C for 5 min and then 5–10 units of restriction enzyme was added and the mix incubated at 37 °C for 1 h. The digestion was terminated by adding Na₂EDTA to 10 mM and SDS to 0.2% and an equal volume of 98% formamide, 10 mM Na₂EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue. It was boiled for 5 min and loaded on a thin denaturing sequencing gel. Single-stranded fragments were eluted from gel slices by soaking overnight at 37 °C in 0.2 ml of 0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1 mM EDTA, 0.1% SDS²⁶. The typical yield was 0.1 pmol or 15 ng of a 450-base fragment. Prime-cut probes synthesized in this way have an average specific activity of 6×10^7 d.p.m. per μ g and are stable for several weeks. **C_{κ} probe:** The template M13/ $C_{\kappa}6$ contains a 469-base fragment starting at codon 115 and extending 170 bases into the 3'-untranslated region. This fragment was inserted into the *HindIII* site of *mp7*. The probe was prepared by cutting with *SacI* which cleaves between codons 201 and 202. It contained 208 bases of C_{κ} sequence and 35 bases of M13 sequence at the 5' end. **$V_{\kappa}1$ probe:** The template M13 101/320 contains a 326-base *RsaI*-*PstI* fragment of the *HK101* gene inserted into the *SmaI*-*PstI* site of *mp8*. This fragment contains 77 bases of the intron and extends to codon 79. The probe was prepared by cutting with *EcoRI* which cleaves in the M13 polylinker seven bases from the end of the insert. It contained 249 bases of immunoglobulin-coding sequences and 112 bases of M13 and intron sequences at the 5' and 3' ends. **$V_{\kappa}3$ probe:** The template M13 *NG9/10* is a subclone of *NG9/3* which contains a 243-base fragment extending from the *HindIII* site at codon 4 to codon 84 cloned into the *SmaI*-*BamHI* site of *mp8*. The probe was prepared by cutting with *PvuII* which cleaves the M13 vector 183 bases from the end of the insert. It contained 243 bases of immunoglobulin-coding sequence and 232 bases of M13 sequence at the 5' and 3' ends. **Hybridization and *exoVII* digestion:** 3 μ g of unfractionated spleen RNA or 1 μ g of PBL RNA was hybridized to 0.008 pmol (a 10-fold molar excess over κ mRNA in spleen) of prime-cut probe (0.5–2 ng) in 5 μ l of 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 10 mM Na₂EDTA at 67 °C for 24 h in a sealed capillary. When a mixture of the two V_{κ} probes ($V_1 + V_3$) was used, 0.004 pmol of each was added. The hybrids were digested in 100 μ l of the same solution containing 4 U ml⁻¹ of *exoVII* (BRL) for 1 h at 37 °C. The reaction was terminated by ethanol precipitation and the products prepared for electrophoresis on a sequencing gel.



are subgroup 3⁵. In agreement with these values, the *NG9* $V_{\kappa}3$ DNA probe hybridized to 22% and 30% of spleen RNA in two individuals. Subgroup 1 constituted 28% and 25% of serum κ chains in two studies^{13,14} while 44% (81/182) of myeloma chains belong to this subgroup⁵. These values agree with frequencies of 39% and 23% obtained by hybridization of the *HK101* $V_{\kappa}1$ gene to spleen RNAs. In short, the data show that spleen mRNA is a faithful representation of the κ -chain repertoire at least insofar as the contribution of the different subgroups is concerned. The agreement of the $V_{\kappa}1$ and $V_{\kappa}3$ frequencies determined by protein analysis and by DNA-RNA hybridization, suggests that the two cloned genes hybridize with nearly all the expressed subgroups 1 and 3 V region sequences. This conclusion would not be valid if each of the V probes by chance hybridized to transcripts from subsets of $V_{\kappa}1$ and $V_{\kappa}3$ genes that were expressed to a disproportionately large extent so as to coincide fortuitously with the protein data. Barring this highly unlikely possibility, the results show that close to all subgroup 1 and subgroup 3 V -region sequences are encoded in the germ-line by only about 25 genes.

The quantitation of V_{κ} sequences in lymphocyte RNA agrees with previous gene-counting experiments which showed that four quite distantly related human and mouse V_{κ} probes all hybridized to the same small family of V genes in human DNA³. Based on the extent of cross-hybridization observed among human V_{κ} sequences, it was argued that the 25 germ-line V_{κ} genes detected, constituted a large proportion of the total V_{κ} gene pool. It has now been demonstrated that this family of genes encodes over 50% of κ mRNA in human lymphocytes. Together, these data suggest that the total number of V_{κ} genes in the human germ line is 50 or fewer. Cory *et al.*¹⁵ estimated

that the most probable number of V_{κ} genes in the mouse was 90, based on statistical analysis of Southern blots hybridized to 10 different V_{κ} sequences. This result is consistent with the fact that the $V_{\kappa}21$ group encoded by six to eight germ-line genes¹⁶ comprises 8% of serum light chains¹⁷. Thus, the number of V_{κ} genes in the mouse germ line may also be quite small although greater than in the human germ line. Although the overall contribution of somatic mutation to the murine antibody repertoire is unknown, several examples of somatic mutation of individual V genes have been documented (see, for example, refs 18–20).

Comparison of the number of germ-line V_{κ} genes with the number of κ chains expressed by an individual allows one to assess the relative importance of the germ-line and somatic components of diversity. The number of different κ variable regions expressed in an individual is not known but it may be approximated by the repertoire of myeloma chains in the human population. Because of polymorphism, the myeloma model gives a maximum estimate for the repertoire of an individual. Quatrocchi *et al.*²¹ analysed 52 myeloma proteins by peptide mapping and found that no two were identical. Also, 36 κ V regions of myeloma chains have been fully or almost fully sequenced⁵ and no two have identical amino acid sequences up to position 95. These observations imply that in addition to the contribution of V - J joining, the number of V_{κ} gene-encoded sequences (amino acids 1–95) among myeloma chains is²¹ at least 1,000. Direct estimates of the number of different antibodies expressed by individual mice predict a repertoire of several thousand κ -chains¹ and it is hard to see why the human repertoire should be very much smaller. It is likely, therefore, that the repertoire of V_{κ} gene-encoded sequences in an individual human is at least

several hundred. The small number of germ-line V_{κ} sequences apparently cannot account for the repertoire without extensive diversification by somatic mutation.

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1. Sigal, N. & Klinman, N. *Adv. Immun.* **26**, 255–337 (1978).
2. Tonegawa, S. *Nature* **302**, 575–581 (1983).
3. Bentley, D. & Rabbitts, T. *Cell* **24**, 613–623 (1981).
4. Milstein, C. *Nature* **205**, 1171–1173 (1965).
5. Kabat, E., Wu, T., Bilofsky, H., Reid-Miller, M. & Perry, H. *Sequences of Proteins of Immunological Interest* (US Dept of Health & Human Services, Washington DC, 1983).
6. Milstein, C. & Pink, J. *Prog. Biophys. molec. Biol.* **21**, 209–263 (1970).
7. Zeelon, E., Bothwell, A., Kantor, F. & Schechter, I. *Nucleic Acids Res.* **9**, 3809–3819 (1981).
8. Bentley, D. & Rabbitts, T. *Nature* **288**, 730–733 (1980).
9. Galfre, G. & Milstein, C. *Immunology* **45**, 125–128 (1982).
10. Bentley, D. & Rabbitts, T. *Cell* **32**, 181–189 (1983).
11. Bentley, D. thesis, Cambridge Univ. (1982).
12. Solomon, A. & McLaughlin, C. *J. exp. Med.* **130**, 1295–1311 (1969).
13. Grant, J. & Hood, L. *Immunochimistry* **8**, 63–79 (1971).
14. Milstein, C., Milstein, C. & Feinstein, A. *Nature* **221**, 151–154 (1969).
15. Cory, S., Tyler, B. & Adams, J. *J. molec. appl. Genet.* **7**, 103–116 (1981).
16. Valbuena, O., Marcu, K., Weigert, M. & Perry, R. *Nature* **276**, 780–784 (1978).
17. Julius, M., McKean, D., Potter, M. & Weigert, M. *Molec. Immun.* **18**, 11–17 (1981).
18. Weigert, M., Cesari, I., Yonkovich, S. & Cohn, M. *Nature* **228**, 1045–1047 (1970).
19. Bernard, O., Hozumi, N. & Tonegawa, S. *Cell* **15**, 1133–1144 (1978).
20. Selsing, E. & Storb, U. *Cell* **25**, 47–58 (1981).
21. Quattrocchi, R., Cioli, D. & Baglioni, C. *J. exp. Med.* **130**, 401–415 (1969).
22. Sanger, F., Nicklen, S. & Coulson, A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
23. Wahl, G., Stern, M. & Stark, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683–3687 (1979).
24. Thomas, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
25. Duckworth, M. *et al. Nucleic Acids Res.* **9**, 1691–1706 (1981).
26. Maxam, A. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
27. Auffray, C. & Rougeon, F. *Eur. J. Biochem.* **107**, 303–314 (1980).

A lymphocyte-specific enhancer in the mouse immunoglobulin κ gene

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During differentiation of lymphocytes into antibody-producing cells, an immunoglobulin κ variable-region gene is transcriptionally activated by rearrangement linking it to a κ constant (C_{κ}) region gene which is already transcribed prior to somatic rearrangement^{1,2}. The presence of a transcriptional enhancer element within the large intron of the κ light-chain gene has been postulated to explain this mode of activation, supported by evidence of a chromatin region which is preferentially accessible to DNase I^{3–5} and restriction enzymes⁵. This DNA region contains a segment of about 130 base pairs (bp) which is strongly conserved between mouse, rabbit and man⁶. Moreover, no transcripts are detectable from a κ gene, which is truncated within the large intron⁷. Recently, a lymphocyte-specific enhancer has been identified downstream of the joining region in immunoglobulin heavy-chain genes^{8–10}. We now show direct evidence for a functionally similar enhancer within the large κ gene intron of the mouse. It is, however, less active than the heavy-chain gene enhancer. In contrast, no enhancer was found to be associated with a cloned λ light-chain gene.

Several DNA fragments from both the κ light-chain gene¹¹ and the λ light-chain gene^{12,13} from the mouse were subcloned into a plasmid carrying the rabbit β -globin gene (Fig. 1). First we took the 2.8-kilobase (kb) *HindIII*–*Bam*HI fragment, which contains the 3' half of the large intron (IVS2) and the entire constant coding region including 3'-flanking sequences of the κ gene, and inserted it downstream of the β -globin gene to yield the recombinant $p\beta\kappa$ D (Fig. 1a). The 7.4-kb *Eco*RI–*Eco*RI segment with the somatically rearranged λ -chain gene was sub-

cloned as *Eco*RI–*Kpn*I fragments to give the recombinants $p\beta\lambda$ U and $p\beta\lambda$ (Fig. 1b).

To study the transient expression of the β -globin gene these recombinants were transfected into mouse myeloma cells (cell line X63Ag8) by the DEAE-dextran technique. As a positive control the recombinant p TAR7 was transfected in parallel. This clone contains the immunoglobulin heavy-chain gene enhancer inserted downstream of the rabbit β -globin gene⁸. The cells were collected 40 h after transfection and the cytoplasmic RNA was analysed by a S_1 nuclease mapping experiment (Fig. 2a). Both $p\beta\kappa$ D, containing the κ gene fragment, and p TAR7 give rise to correctly initiated β -globin gene transcripts, although the level of transcription from $p\beta\kappa$ D is considerably lower (Fig. 2b). The finding of transcripts from $p\beta\kappa$ D is consistent with a recent report showing that deletion of the 2.8-kb *HindIII*–*Bam*HI fragment abolishes expression of the κ gene when this truncated gene is introduced into myeloma cells⁷. The recombinants with the λ gene fragments ($p\beta\lambda$ U and $p\beta\lambda$) gave globin gene transcripts neither in the κ chain-producing myeloma line X63Ag8 (Fig. 2b) nor in the myeloma line J558 (data not shown), which produces λ I chains. Interestingly, even transcription of the intact λ gene transfected into myeloma cells is only detectable when the gene is linked to the simian virus 40 (SV40) or heavy-chain gene enhancer (unpublished results). This parallels the expression of the λ gene alone or linked to the SV40 enhancer on transfection into human HeLa cells¹⁴.

To narrow down the region of the κ gene responsible for the enhancing effect, we subcloned DNA fragments from the DNase I hypersensitive region found in the large κ gene intron^{3–5} (Figs 1a, 3a). These subfragments do not include the constant coding region, polyadenylation site or 3'-flanking sequences. The clone $p\beta\kappa$ D1 contains the 266-bp *Alu*I–*Hae*III fragment inserted behind the rabbit β -globin gene. In clone $p\beta\kappa$ D11 the κ gene fragment extends 209 bp further to the *Alu*I site and thus includes the entire segment of about 130 bp from the large κ gene intron (Fig. 3b), which is strongly conserved between mouse, rabbit and man (' κ intron conserved region')⁶. In clones $p\beta\kappa$ D2 and $p\beta\kappa$ D22 the inserts are in opposite orientation. These recombinants were also transfected into myeloma cells, and the cytoplasmic RNA was analysed by S_1 mapping. This experiment shows enhancement of β -globin gene transcription by the 475-bp κ gene fragment in either orientation (Fig. 2c, lanes 2, 3), whereas removal of 209 bp from the *Hae*III to the *Alu*I site abolishes the effect (Fig. 2d). The deletion removes about 60% of the conserved segment (Fig. 3b). Note that the 475-bp fragment still retains the full enhancement activity as compared with the 2.8-kb fragment in $p\beta\kappa$ D (Fig. 2c).

It is interesting to consider a few features of the enhancing DNA sequence (Fig. 3b). As DNase I hypersensitivity sites are known to be closely associated with the enhancer elements of polyoma virus¹⁵ and SV40¹⁶, it is noteworthy that the inactive 266-bp κ gene intron segment retains only part of the chromatin region accessible to nucleolytic enzyme cleavage. The stretches of sequence homology^{4,6} between the inactive 266-bp segment and the SV40 72-bp repeat or the polyoma enhancer segment are apparently not sufficient for enhancement. It remains to be determined whether the κ intron conserved region contains all of the enhancer activity. The κ gene enhancing element also cannot act in *trans* because co-transfection of a globin gene recombinant and the κ gene fragment subcloned in pUC8 does not yield detectable globin gene transcripts (Fig. 2c, lane 4). This is corroborated by the finding that the κ gene enhancer fragment does not have long open reading frames and is not detectably transcribed into an independent RNA⁶. We also conducted a transient expression assay with mouse fibroblast 3T6 cells. In contrast to a globin gene recombinant with the SV40 enhancer, $p\beta\kappa$ D22 did not give globin gene transcripts (data not shown). This indicates that the κ gene enhancer is lymphocyte specific, as was shown for the heavy-chain gene enhancer⁸.

The κ gene enhancer shares common features with the previously characterized viral^{17–19} and immunoglobulin heavy-

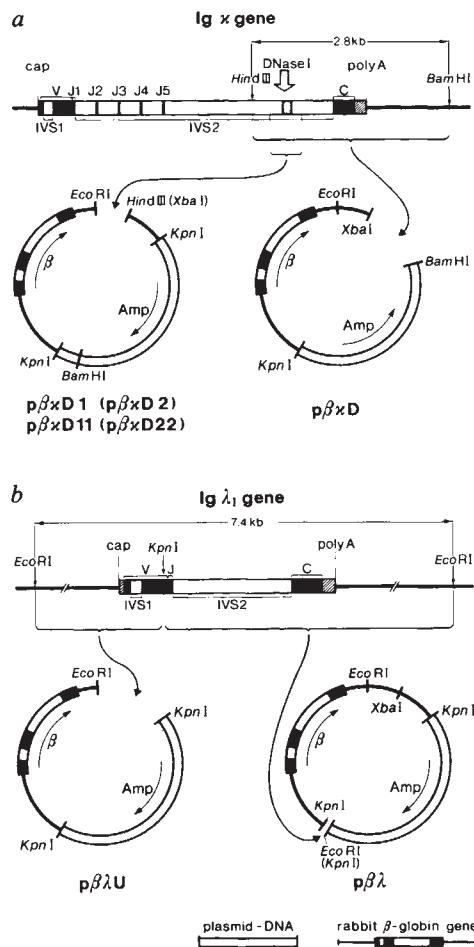


Fig. 1 Schematic representation of recombinant DNAs used. *a*, Diagram of the κ light-chain gene from the mouse myeloma MOPC-41^{11,25}. The open arrow indicates the centre of the DNase I hypersensitivity site within IVS2³⁻⁵ positioned according to ref. 4. The stippled box represents the κ intron conserved region (ref. 6, see also Fig. 3). Scheme of the strategy applied to subclone κ gene fragments into the rabbit haemoglobin β 1 gene²⁶ recombinants p β GX and p β G- (ref. 8). p β κ D: 2.8-kb *Hind*III-*Bam*HI κ gene fragment cloned into p β G- by fusing *Hind*III and *Xba*I sites. p β κ D1/p β κ D11: the small κ gene fragments indicated in Fig. 3 were first subcloned in either orientation into the polylinker of plasmid pUC8²⁷ and thereafter excised as *Eco*RI-*Hind*III fragments to be inserted into p β GX. Clones p β κ D1 and p β κ D11 thus contain the 266-bp *Alu*I-*Hae*III and the 475-bp *Alu*I-*Alu*I intron fragments (see also Fig. 3), respectively. p β κ D2/p β κ D22: reversed orientation of 266-bp and 475-bp intron fragments, respectively. *b*, Diagram of the mouse λ 1 light-chain gene^{12,13}. p β λ U/p β λ : the two fragments generated by a partial *Kpn*I digestion of the 7.4-kb *Eco*RI fragment were inserted into the rabbit β -globin gene recombinant p β G⁸. V, J and C, variable, joining and constant regions of immunoglobulin genes. IVS1 and IVS2, intervening sequences 1 and 2.

chain gene enhancers⁸⁻¹⁰. It also acts in an orientation-independent manner over a large distance and only works in *cis*. Although there are no extended sequence homologies between the large intron of κ and heavy-chain genes⁶, we consider it likely the κ gene fragment acts in the same way as the heavy-chain gene enhancer⁸ and not, for example, as an uncontrolled origin of DNA replication which amplifies template copy number. Both immunoglobulin gene enhancers show a remarkable cell-type specificity. However, the κ gene enhancer is only about 5% as active as the heavy-chain gene enhancer (Fig. 2*d*) in our assays. Trivial explanations such as the quality of the DNA preparation have been excluded: the same results were obtained in several *S*₁ nuclease mapping experiments using independent

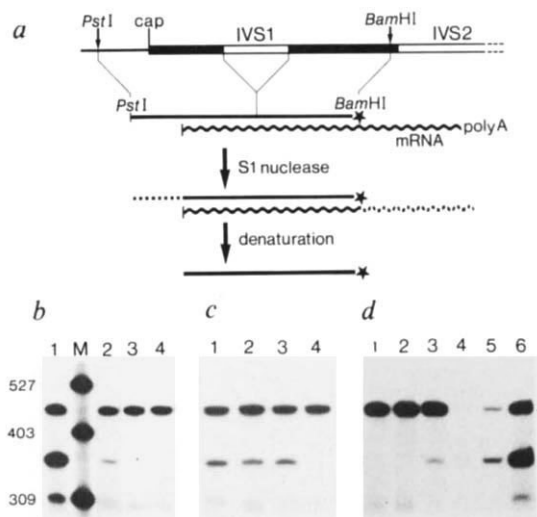


Fig. 2 Enhanced β -globin gene transcription. Tissue culture cell lines were grown and transfected as described earlier⁸. Total cytoplasmic RNA was extracted from the myeloma cells X63Ag8 40–42 h after transfection and incubated with RNase-free DNase¹⁴ to remove residual input plasmid DNA. The recombinant pTAR7 used as a positive control contains the heavy-chain gene *Xba*I enhancer fragment cloned into the *Xba*I site of the rabbit β -globin gene recombinant p β G- (ref. 8, see also Fig. 1 legend). *a*, *S*₁ nuclease mapping scheme²⁸. A β -globin gene clone lacking the first intervening sequence (IVS1)²⁹ was used to prepare the radioactive probe³⁰. DNA end-labelled at the *Bam*HI site was hybridized to unlabelled RNA, treated with *S*₁ nuclease, denatured, fractionated by gel electrophoresis and autoradiographed (see ref. 17). *b*, Hybridization to 50 μ g RNA from X63Ag8 myeloma cells transfected with recombinant pTAR7 (lane 1), p β κ D (lane 2), p β λ (lane 3) and p β λ U (lane 4). M, end-labelled marker DNA fragments (sizes in nucleotides) of pBR322 digested with *Hpa*II. *c*, Hybridization to 70 μ g RNA from X63Ag8 myeloma cells transfected with recombinant p β κ D1 (lane 1), p β κ D2 (lane 2) and p β κ D11 (lane 3). The DNA probe protected from *S*₁ nuclease cleavage after hybridization to 90 μ g RNA from X63Ag8 cells transfected with pTAR7 was applied to gel electrophoresis in three dilutions: lane 4, 1:50; lane 5, 1:10; lane 6, remaining 80% of protected DNA. By comparing lanes 3 and 5, the relative ratio of β -globin gene transcripts from recombinants p β κ D11 (κ gene enhancer) and pTAR7 (heavy-chain gene enhancer) was estimated to be about 1:20. fl, Full-length probe (453 nucleotides); ct, fragment with correct terminus, mapping 354 nucleotides upstream from the *Bam*HI site.

DNA preparations; neither is it due to a deficiency of the particular myeloma line used (X63Ag8). The κ gene enhancer was also comparatively weak when pTAR7 and p β κ D were transfected into the λ chain-producing myeloma J558 (not shown). The same ratio of enhancer activity was observed when the two enhancers were linked to an enhancerless SV40 T-antigen gene, and gene expression in myeloma X63Ag8 and hybridoma Sp6BU6BU was measured by immunofluorescence (not shown). Furthermore, no stimulation of κ gene enhancer activity was seen on incubation of transfected myeloma cells with bacterial lipopolysaccharide (data not shown), which in certain cells of the B-lymphocyte lineage induces the DNase I hypersensitivity region in the κ gene intron and stimulates light-chain gene transcription^{3,5}.

It therefore appears paradoxical that both κ and heavy-chain gene mRNAs accumulate to about the same level, have a similar turnover in myeloma cells²⁰, and yet the genes have enhancers of different strength. It seems possible that the low activity of the κ gene enhancer relative to the heavy-chain gene enhancer is compensated by additional transcriptional regulatory elements

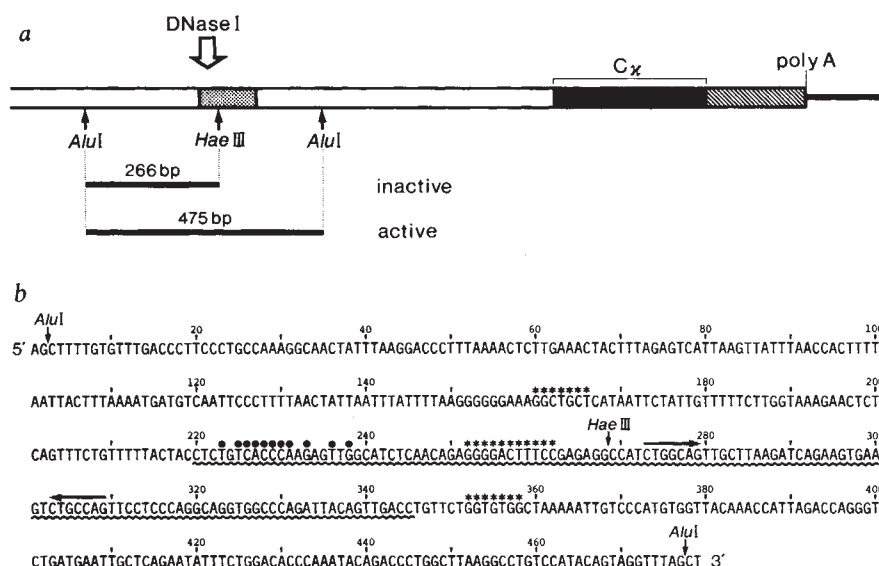


Fig. 3 κ enhancer fragments and nucleotide sequence. *a*, Enlarged schematic representation (see also Fig. 1) of κ light-chain intron (IVS2) and constant region (C_κ). Restriction sites used to generate the 266-bp and 475-bp κ enhancer fragments (black bars) are shown. Stippled box, κ intron conserved region⁶. *b*, Nucleotide sequence^{5,25} of the active 475-bp κ enhancer fragment. The wavy line below the sequence represents the κ intron conserved region. Asterisks and dots above the sequence indicate sequence homologies of at least 7 bp to the SV40 (the first two blocks are mentioned in ref. 4) and polyoma virus⁶ enhancer elements, respectively. Arrows indicate a 7-bp inverted repeat sequence⁵. The chromatin region, which is preferentially accessible to cleavage by DNase I³⁻⁵ and various restriction enzymes, roughly maps to the sequence between nucleotides 180 and 420⁵.

5' to the region we have analysed. It could also mean that relative enhancer strength is only crucial in an early stage of B-cell differentiation, for example, to ensure that heavy-chain gene transcription precedes κ gene transcription. At later stages maximal transcription of both gene types could be controlled by factors acting in *trans*. In our assays, limiting factors might remain associated with the endogenous chromosomal genes. Such *trans*-acting factors have been described for 5S RNA gene transcription²¹ and for 'TATA box' recognition²², where they form 'preinitiation complexes' which are highly stable. More recently, *trans*-acting viral proteins have been described which permit efficient transcription of viral and nonviral genes even in the absence of enhancer sequences.²³

This concept could also explain the absence of an enhancer sequence from the λ light-chain gene clone, although the latter finding may be related to the unique structure of murine λ genes²⁴. As there is only one λ variable region for each of the two constant region clusters, an enhancer could also be located far upstream of the variable region.

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1. Mather, E. L. & Perry, R. P. *Nucleic Acids Res.* **9**, 6855-6867 (1981).
2. Van Ness, B. G. *et al. Cell* **27**, 593-602 (1981).
3. Parslow, T. G. & Granner, D. K. *Nature* **299**, 449-451 (1982).
4. Chung, S.-Y., Folsom, V. & Wooley, J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2427-2431 (1983).
5. Parslow, T. G. & Granner, D. K. *Nucleic Acids Res.* **11**, 4775-4792 (1983).
6. Emorine, L., Kuehl, M., Weir, L., Leder, P. & Max, E. E. *Nature* **304**, 447-449 (1983).
7. Queen, C. & Baltimore, D. *Cell* **33**, 741-748 (1983).
8. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729-740 (1983).
9. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717-728 (1983).
10. Neuberger, M. S. *EMBO J.* **2**, 1373-1378 (1983).
11. Seidman, J. G. & Leder, P. *Nature* **276**, 790-795 (1978).
12. Brack, C. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5652-5656 (1977).
13. Bernard, O., Hozumi, N. & Tonegawa, S. *Cell* **15**, 1133-1144 (1978).
14. Picard, D. & Schaffner, W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 417-421 (1983).
15. Herbolme, P., Saragosti, S., Blangy, D. & Yaniv, M. *Cell* **25**, 651-658 (1981).
16. Cremisi, C. *Nucleic Acids Res.* **9**, 5949-5964 (1981).
17. de Villiers, J. & Schaffner, W. *Nucleic Acids Res.* **9**, 6251-6264 (1981).
18. Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299-308 (1981).
19. Moreau, P. *et al. Nucleic Acids Res.* **9**, 6047-6068 (1981).
20. Schibler, U., Marcu, K. B. & Perry, R. P. *Cell* **15**, 1495-1509 (1978).
21. Bogenhagen, D. F., Wormington, W. M. & Brown, D. D. *Cell* **28**, 413-421 (1982).
22. Davison, B. L., Egly, J.-M., Mulvihill, E. R. & Chambon, P. *Nature* **301**, 680-686 (1983).
23. Green, M. R., Treisman, R. & Maniatis, T. *Cell* **35**, 137-148 (1983).
24. Tonegawa, S. *Nature* **302**, 575-581 (1983).

25. Max, E. E., Maizel, J. V. Jr & Leder, P. *J. biol. Chem.* **256**, 5116-5120 (1981).
26. Maniatis, T. *et al. Cell* **15**, 687-701 (1978).
27. Vicaria, J. & Messing, J. *Gene* **19**, 259-268 (1982).
28. Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).
29. Weber, H. *et al. ICN-UCLA Symp. molec. cell. Biol.* **33**, 367-385 (1981).
30. Rusconi, S. & Schaffner, W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5051-5055 (1981).

Conservative segregation of nucleosome core histones

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Density labelling studies have shown that nascent histones are not mixed with parental histones during the assembly of nucleosome cores^{1,2}. However, experiments in other laboratories, examining histone deposition with respect to newly synthesized DNA, have been interpreted as suggesting that a substantial proportion of core histones (>15%) are randomized at each chromatin replication³⁻⁸. The data presented here support our previous results in showing that conservatively assembled nucleosome core histone octamers are conservatively segregated over successive cell generations. It is also shown that the nucleosome cores assembled during 1- β -D-arabinofuranosylcytosine inhibition of DNA synthesis are conservatively segregated for a minimum of five or six cell generations. These results suggest that the nonrandom assembly of nucleosome cores is not merely a coincidence of the mechanism of histone transport into the nucleus and that the conservative mode of nucleosome segregation is a fundamental feature of chromatin replication, one which is stable to modulations in chromatin packaging.

Our laboratory has previously shown that when MSB cells (a line of leukaemic chicken lymphocytes⁹) are incubated for 1 h in medium containing ¹⁵N-, ¹³C-, ²H-substituted amino acids (plus tracer ³H-lysine), the histone synthesized during this incubation (~5-10% of the total histones) bands with a 4-5% increase in density on denaturing isopycnic caesium formate-guanidine hydrochloride (CsFo-GuCl) gradients. By isolation of chromatin immediately after a 1-h density pulse and subsequent cross-linking of nucleosome core histone octamers, it was demonstrated that the ³H-labelled octamers are conservatively assembled; that is, each octamer contains eight density-substituted histones².

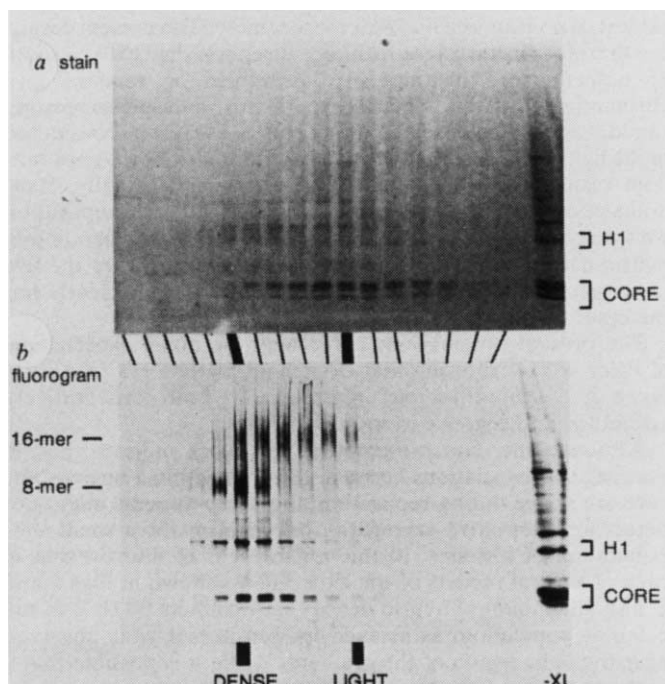


Fig. 1 Conservative segregation of nucleosome core histones. Nucleosome core octamers containing density-labelled, ^3H -histones were separated from unlabelled octamers on CsFo-GuCl. The gradient was analysed by SDS-PAGE. *a*, Coomassie blue stained gel. Note that bulk 8-mers and 16-mers are not readily detected due to the strongly decreased binding of Coomassie blue to chemically cross-linked histones²². *b*, Fluorogram. 'DENSE' ($\rho = 1.30 \text{ g ml}^{-1}$) and 'LIGHT' ($\rho = 1.24 \text{ g ml}^{-1}$) refer to peak positions of uncross-linked, density-substituted, ^3H -labelled histone and cross-linked, light, ^{14}C -labelled histone markers run in a parallel gradient. Lane marked -XL represents uncross-linked marker H1-, non-histone-depleted chromatin before centrifugation.

Methods: Exponentially growing MSB cells were washed with phosphate-buffered saline (PBS) and resuspended in 1 ml ($8 \times 10^6 \text{ cells ml}^{-1}$) of growth medium containing density-substituted amino acids (Merck MB1808) reconstituted to approximate RPMI-1640. After a 15-min preincubation, ^3H -lysine (NEN, 71 Ci mmol^{-1} , $200 \mu\text{Ci ml}^{-1}$) was added for 1 h. The cells were washed with PBS and returned to RPMI-1640 for a further 72 h of culture. Chromatin depleted in H1 and non-histone protein was isolated, an aliquot was cross-linked with dithiobissuccinimidyl propionate and the mixture of cross-linked and uncross-linked chromatin centrifuged to equilibrium on CsFo-GuCl as described previously². The gradients were collected from below and fractions ($65 \mu\text{l}$) microdialysed against SDS-PAGE sample buffer²³ omitting β -mercaptoethanol at all times. Fractions were analysed by 5–22% gradient acrylamide SDS-PAGE²³. The gel was processed for fluorography using Enhance (NEN) and exposed to preflashed²⁴ Kodak X-Omat AR film at -70°C .

The conservative assembly of newly synthesized cores suggests, but does not prove, that the parental histone octamers may be inherited in a nonrandom fashion. To examine directly the stability of core histone octamers, MSB cells were pulsed for 1 h in density-substituted medium (plus tracer ^3H -lysine) and then washed and resuspended in their normal growth medium for an additional five or six cell generations. At this point the ^3H -labelled, density-substituted histone represents less than 1% of the total histone population.

Chromatin was isolated after the density labelling pulse and five to six generation chase and depleted of histone H1 and several non-histone proteins² so as to permit exclusive examination of the core histone octamer. The depleted chromatin was cross-linked with dithiobissuccinimidyl propionate and centrifuged to equilibrium in CsFo-GuCl. On these gradients the DNA pellets, and proteins not cross-linked to one another band

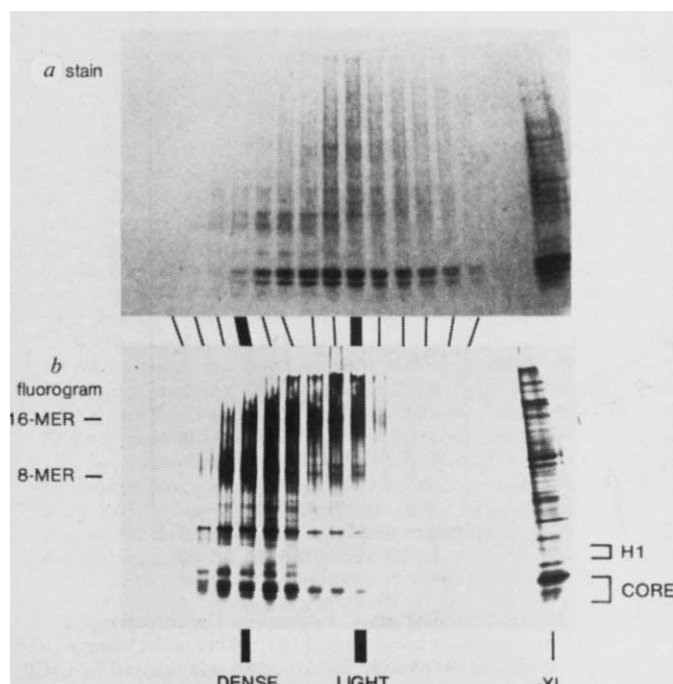


Fig. 2 Conservative segregation of nucleosome cores assembled during ara-C incubation. Octamers containing density-labelled, ^3H -histones synthesized during ara-C incubation were separated from unlabelled histone octamers on CsFo-GuCl. The gradient was analysed by SDS-PAGE. *a*, Stained gel; *b*, fluorogram.

Methods: An exponentially growing culture of cells was incubated for 30 min with ara-C ($50 \mu\text{g ml}^{-1}$), pelleted at low speed and resuspended in density-substituted medium containing ara-C for 30 min. ^3H -lysine was added to $200 \mu\text{Ci ml}^{-1}$ and the incubation continued for 90 min. Cells were pelleted, washed with PBS and returned to fresh RPMI-1640 (no drug) for 72 h of culture. Depleted chromatin was isolated, cross-linked and centrifuged on a CsFo-GuCl gradient as in Fig. 1.

independently. The gradient was fractionated and individual fractions analysed by 5–22% acrylamide gradient SDS-polyacrylamide gel electrophoresis (PAGE). The Coomassie blue stained gel of Fig. 1*a* shows the distribution of bulk (light, parental) histone in the gradient. The fluorogram of this gel (Fig. 1*b*) reveals that the histone octamers labelled with ^3H -lysine before the chase in unlabelled medium still appear as uniformly dense particles, as defined by the position of uncross-linked dense histones run in the same gradient. Furthermore, the density distribution of the ^3H -octamers assayed after the chase is the same as that before the chase^{1,2}. These octamers, therefore, have been conservatively inherited.

When MSB cells are incubated in 10^{-4} M 1- β -D-arabinofuranosylcytosine (ara-C), DNA synthesis is inhibited while core histone synthesis proceeds at $\sim 20\%$ of control levels in 100–200-fold excess over nascent DNA¹⁰. In these conditions, nucleosome cores are assembled conservatively²; however, the structure of the chromatin synthesized during ara-C incubation is altered in that these nucleosomes adopt a shortened repeat length. On release from ara-C inhibition, the repeat length of this chromatin slowly returns to control levels¹⁰. This system has been used to investigate whether the nucleosome histone octamers assembled during ara-C incubation are stably maintained during drug recovery and subsequent cell growth.

MSB cells were incubated in 10^{-4} M ara-C for 2 h in the presence of density-substituted medium plus ^3H -lysine tracer, washed and then transferred to normal growth medium for an additional five or six cell generations. Depleted chromatin was isolated, cross-linked as described in Fig. 1 legend and centrifuged to equilibrium in CsFo-GuCl. The stained gel of Fig.

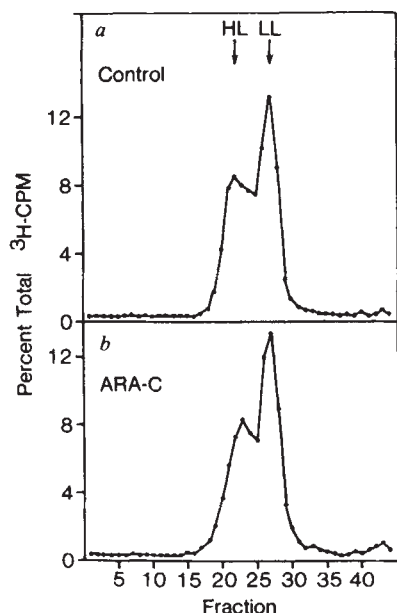


Fig. 3 Replication after ara-C incubation. The incorporation of bromodeoxyuridine by cells labelled 18 h earlier under control (a) or ara-C incubations (b) with ^3H -thymidine was assayed by CsCl density gradient centrifugation.

Methods: An exponentially growing cell culture was split 1:1 with fresh RPMI-1640 and incubated with or without ara-C ($50 \mu\text{g ml}^{-1}$) for 1 h. The cultures were concentrated to 1 ml (10^7 cells ml^{-1}) and incubated with ^3H -thymidine (NEN, 24 Ci mmol^{-1}) at $5 \mu\text{Ci ml}^{-1}$ (control) or $200 \mu\text{Ci ml}^{-1}$ (ara-C culture), for 2 h. Each culture was pelleted, washed and resuspended in warmed RPMI-1640 containing $5 \mu\text{g ml}^{-1}$ thymidine in the presence (ara-C culture) or absence (control) of ara-C for an additional hour. Cultures were pelleted and resuspended in fresh RPMI-1640 in the absence of ara-C for 18–20 h. Bromodeoxyuridine (Sigma) was added to each culture to $50 \mu\text{g ml}^{-1}$ and the cells were collected after 3 h. DNA was prepared by treatment of isolated nuclei with 10 mM Na_2EDTA , 0.2% SDS, protease K ($200 \mu\text{g ml}^{-1}$, 3 h, 37°C), phenol extraction and ethanol precipitation. The DNA was sheared by three passages through a 26-gauge syringe needle, 50 μl mixed with 2.8 ml of 5.93 M caesium chloride and centrifuged in the Beckman SW60 Ti rotor at 30,000 r.p.m. for 48 h. Fractions were collected from below and counted in Beckman Ready-Solv MP. Arrows indicate positions of light (LL) and hybrid (HL) density markers run in parallel. (Control experiments (not shown) demonstrated that greater than 70% of the ^3H -thymidine in b was incorporated during the ara-C incubation.)

2a shows the banding pattern of the bulk histones while the fluorogram of Fig. 2b demonstrates that the ^3H -labelled histones synthesized during the ara-C incubation comprise uniformly dense octamers after the five to six generation chase in normal medium.

Control experiments (not shown) indicate that >85% of the ^3H -histone incorporation occurred during the initial ara-C incubation and that >90% of the ^3H -histones are retained after five or six cell doublings.

To show that MSB cells are not precluded from subsequent replications as a result of ara-C incubation, cells were incubated in the presence of ^3H -thymidine for 2 h with or without ara-C. After washing and resuspension in drug-free medium overnight, the cells were transferred to medium containing bromodeoxyuridine for 3 h. Figure 3 demonstrates that after the bromodeoxyuridine pulse ~30% of the total cellular DNA is found to exhibit a heavy–light density. (Similar results are obtained when the initial incubation is performed in dense amino acid medium with or without ara-C (not shown).) This is the amount of hybrid density DNA expected based on the fraction of cells in S phase during the bromodeoxyuridine pulse. Therefore, the nucleosome cores assembled during ara-C incubation are able to replicate thereafter, and the histone octamer is stably inherited.

The conservative segregation of histones is a stringent biological test of nonrandom nucleosome assembly. The present results are therefore inconsistent with recent reports that 15% or more of nascent core histones are assembled at random into chromatin^{3–8}. In this type of experiment, a result suggesting random deposition or segregation of histones must be considered in the light of undiscerned artefactual randomization. A nonrandom result, however, cannot have arisen artefactually. Randomization of 15% of the core histones at each replication would result in >75% of the ^3H -labelled histones banding in hybrid density particles close to the light marker after the five to six generation chase in our experiments. This is clearly not the case.

The present observations agree with the novel experiments of Prior *et al.*¹¹ showing that exogenous histone H3 molecules taken up by *Physarum* microplasmodia are both conservatively assembled and segregated in nucleosomes.

Although the data presented in this work indicate that, in general, the associations between histones within a nucleosome core are stable during replication, these experiments might not detect an alternative segregation mechanism for a small subpopulation of histones. In this regard, it is of interest that in each of several repeats of the experiments shown in Figs 1 and 2, a small amount of hybrid density ^3H -octamers ($\leq 10\%$ of the octamer population, as assayed by densitometry) is observed near the light region of the gradient. While it is possible that a variable 1–2% of the total ^3H -histones segregates randomly at each replication, the conservative assembly^{1,2} and segregation of the vast majority of histone suggest that it may be more likely that a larger, invariant subpopulation of the ^3H -histones ($\approx 10\%$) segregates following a nonrandom pattern which is distinct from that of bulk histone.

These experiments do not directly address the question of the stability of the interaction between a core histone octamer and a particular DNA strand. Several reports have suggested that during replication parental histones may be nonrandomly segregated with respect to one or the other DNA strand^{12–19} (but see ref. 20). The present data are not inconsistent with these observations.

The fluorogram of Fig. 1b indicates that after five or six cell generations there has been no mixing of new and old histones within individual octamers; however, most of the 16-mer particles band with hybrid density. In contrast, when 16-mers are examined immediately after deposition, no more than 10–20% show a hybrid density (compare Fig. 2, ref. 2). If the 16-mer particles result from the cross-linking of linearly contiguous octamer cores, the present data suggest that, as a result of repeated replication in normal medium, most dense octamers have come to be alongside light octamers. This situation could arise if individual nucleosomes segregated randomly (with respect to DNA strand) or if a domain of oligonucleosomes segregated conservatively²¹ but the boundaries of such a domain were variable.

When DNA synthesis is inhibited by ara-C the deposition of newly synthesized histone octamers is accompanied by a decrease in the nucleosome repeat length¹⁰. The chromatin is subsequently remodelled such that the repeat length returns to normal. The results presented here show that the integrity of the octamer core is stable despite repositioning with respect to DNA sequence and modulation of chromatin packaging.

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1. Leffak, I. M., Grainger, R. & Weintraub, H. *Cell* **12**, 837–845 (1977).
2. Leffak, I. M. *Nucleic Acids Res.* **11**, 2717–2732 (1983).
3. Russek, G. & Hancock, R. *Nucleic Acids Res.* **9**, 4129–4137 (1981).
4. Hancock, R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2130–2134 (1978).
5. Jackson, V., Marshall, S. & Chalkley, R. *Nucleic Acids Res.* **9**, 4563–81 (1981).
6. Jackson, V. & Chalkley, R. *Cell* **23**, 121–134 (1981).
7. Annunziato, A. T., Schindler, R. K., Riggs, M. K. & Seale, R. L. *J. biol. Chem.* **257**, 8507–8515 (1982).

8. Jackson, V. & Chalkley, R. J. *biol. Chem.* **256**, 5095-5103 (1981).
9. Akiyama, Y. & Kato, S. *Biken J.* **17**, 105-116 (1974).
10. Leffak, I. M. *Nucleic Acids Res.* **11**, 5451-5466 (1983).
11. Prior, C. P., Cantor, C. R., Johnson, E. V. & Allfrey, V. G. *Cell* **20**, 597-608 (1980).
12. Weintraub, H. *Cold Spring Harb. Symp. quant. Biol.* **38**, 247-256 (1973).
13. Weintraub, H. *Cell* **9**, 419-422 (1976).
14. Seale, R. L. *Cell* **9**, 423-429 (1976).
15. Freedlander, E. F., Taichman, L. & Smithies, O. *Biochemistry* **16**, 1802-1808 (1977).
16. Riley, D. & Weintraub, H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 328-332 (1979).
17. Seidman, M. M., Levine, A. J. & Weintraub, H. *Cell* **18**, 439-450 (1979).
18. Roufa, D. *Cell* **13**, 129-138 (1978).
19. Roufa, D. & Marchionni, M. A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1810-1814 (1982).
20. Fowler, E., Farb, R. & El-Saidy, S. *Nucleic Acids Res.* **10**, 735-748 (1982).
21. Pospelov, V., Russev, G., Vassilev, L. & Tsanev, R. J. *molec. Biol.* **156**, 79-91 (1982).
22. Leffak, I. M. *Analyt. Biochem.* (in the press).
23. Laemmli, U. *Nature* **227**, 680-685 (1970).
24. Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335-341 (1975).

Timing of the steps in transformation of C3H 10T $\frac{1}{2}$ cells by X-irradiation

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Transformation of cells in culture by chemical carcinogens or X rays seems to require at least two steps. The initial step is a frequent event; for example, after transient exposure to either methylcholanthrene or X rays, almost every cell of established lines of mouse embryo fibroblasts proved capable of yielding transformed, tumorigenic descendants¹⁻³. Although results were interpreted as indicating that 100% of the progeny of methylcholanthrene-treated cells were potentially transformed¹, later experiments showed that only a very small minority of the progeny of cells initiated by X rays² or methylcholanthrene³ actually produced transformed colonies. We thus concluded² that there must be a second step in transformation that is a very rare event. We assumed that this event occurred after the cultures became confluent, a time when transformed cells have a selective growth advantage. Since then, however, others have shown that transformation can occur soon after initiation⁴ and that clones of transformed cells may already be present by the time initiated cultures become confluent⁵. It has been hypothesized that the second step behaves like a spontaneous mutation in having a constant but small probability of occurring each time an initiated cell divides^{3,6}. We show here that the clone size distribution of transformed cells in growing cultures initiated by X rays is, indeed, exactly what would be expected on that hypothesis.

If events such as mutations are scattered at random throughout the lineage of a growing population of cells, the number of mutants finally present will vary greatly from one culture to another; this is the basis for the Luria-Delbrück fluctuation test⁷. With a mutation rate of μ per cell generation, populations undergoing binary fission will on the average have experienced $2\mu N$ mutations by the time they contain N cells, namely, μN events in the last generation, $\mu N/2$ in the previous generation, and so on to give a total of $2\mu N$. The proportion of such populations that have not had an event and therefore contain no mutants will simply be $P_0 = e^{-2\mu N}$. Because clones that contain a single mutant cell can have arisen only among the final generation of cells, the proportion of cultures that contain exactly one mutant will be the proportion that had no mutations before the last generation and exactly one mutation in the last generation, that is, $P_1 = e^{-\mu N}(\mu N e^{-\mu N}) = \mu N e^{-2\mu N}$. Unfortunately, the expression for the higher terms of the distribution is more complex because any particular number of mutants could have been achieved in several ways; for example, there could be two mutants as the result of two events in the final generation or one event in the previous generation; and as the number of mutants becomes larger, so the ways of achieving that number become more numerous. But the higher

Table 1 Number of transformed cells in cultures exposed to 600 rad, grown to confluence and then reseeded

Treatment group	No. of transformed foci in each dish
Group I	0, 0, 0, 0, 0, 0, 0, 0, 0, 0
28 cultures, not reseeded	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 2, 2, 2, 3, 3, 4, 4, 4
Group II	
27 cultures, each totally reseeded on to a single dish	0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 1, 1, 2, 2, 3, 4, 4, 5, 5, 7, 12, 19
Group III	
14 cultures, each totally reseeded on to two dishes	(a) 0, 0, 0, 0, 1, 1, 1, 1, 2, 0, 0, 1, 6, 40 (b) 0, 0, 0, 0, 0, 1, 2, 4, 5, c, c, c, c, c, c
Group IV	
28 cultures, each 80% reseeded on to one dish	0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 2, 2, 2, 2, 2, 3, 3, 4, 4, 5, 5, 5, 13, 15, 22

Stock cultures of C3H 10T $\frac{1}{2}$ cells were maintained in 60-mm Petri dishes and were passed by subculturing at a 1:20 dilution every 7 days. The cells used in this experiment were in passage 9. Cells were grown in a humidified 5% CO₂ atmosphere at 37°C in Eagle's basal medium supplemented with fetal calf serum (10% for the first 2 weeks of the transformation assay period, and 5% for the last 4 weeks) and gentamycin (5 μ g ml⁻¹). Dishes were seeded with 12,000 cells and 24 h later were irradiated with 600 rad. As the plating efficiency at this stage in the experiment was 20% and the survival after 600 rad is ~10%, each dish started with an average of 240 surviving irradiated cells. In group I the dishes were not trypsinized. In group II, the cultures were trypsinized as they approached confluence and the cells were then reseeded into their original dishes. In group III, the cultures were trypsinized and reseeded into two separate dishes (a and b). In group IV, the cells were treated as in group II, except that 20% of the trypsinized suspension was removed to form part of a separate experiment. In the paired sample of group III, c indicates that the culture was lost due to contamination; in groups I, II and IV, dishes lost due to contamination are not included. Types 2 and 3 transformed foci were scored as previously described². When cells are re-plated at the high cell densities used here, the plating efficiency is assumed to be at least 50%. We have previously observed that about 50% of cells transformed by X-irradiation will grow, and form transformed foci when reseeded onto confluent monolayers of untreated C3H10T $\frac{1}{2}$ cells¹⁵.

terms can be obtained from Lea and Coulson⁸ who have tabulated the value of P_r for different values of r and $2\mu N$ (which they call m).

To determine the actual distribution of transformed clone sizes, cultures of C3H 10T $\frac{1}{2}$ cells were irradiated with 600 rad of X rays, a dose that kills 90% of the cells, and initiates virtually all those that survive². After growth of the surviving cells to near confluence (about 13 generations), each culture was trypsinized, suspended and totally reseeded on to one or more plates that were maintained for 4 additional weeks, at which time the foci of transformed cells were counted; at the same time, some plates were not reseeded, but were left untouched.

As Table 1 shows, the process of trypsinization and reseeded did not produce any change in the proportion of cultures having one or more transformed foci. Roughly one-third of the cultures yielded no transformed foci. Therefore, $2\mu N$ should be approximately equal to one. Table 2 compares the observed distribution of transformed clone sizes with the values expected on the assumption that the rare step in transformation occurs at random during exponential growth of the progeny of initiated cells and that there is, on the average, one such event per culture. As the differences between observed and expected are not significant, it seems that the second step is indeed occurring at random. The stages in such *in vitro* transformation can therefore be summarized as follows.

Although the initial step is driven by mutagenic carcinogens and creates a fairly stable heritable state, it is unlikely to be a mutation because a single treatment with X rays or methylcholanthrene can initiate almost every cell^{1-3,9}. Furthermore, lower doses of initiator seem to place cells in an intermediate

Table 2 Comparison of expected and observed distributions of clone sizes for the data presented in Table 1

	No. of transformed colonies in reseeded groups (<i>r</i>)								
	0	1	2	3-4	5-8	9-16	17-32	33-64	>64
Probability that a culture has <i>r</i> transformants (mutants) when the average number (<i>m</i>) of events (mutations) per culture is 1 (ref. 8, Table 2)	0.3679	0.1839	0.1073	0.1164	0.0961	0.0617	0.0335	0.0168	0.0163
Data for 36 reseeded cultures of groups II and III (i)									
Observed no. of dishes containing <i>r</i> transformed foci	13	9	3	4	5	1	1	0	0
Expected no., from the above probabilities	13.2	6.6	3.9	4.2	3.5	2.2	1.2	0.6	0.6
Data for 69 reseeded dishes of groups II-IV (ii)									
Observed no. of dishes containing <i>r</i> transformed foci	23	14	9	8	8	4	2	0	1
Expected no., from the above probabilities	25.4	12.7	7.4	8.0	6.6	4.3	2.3	1.2	1.1

Comparison (i) excludes those pairs of dishes in group III where one dish was contaminated. The clone size for the uncontaminated pairs was taken to be the sum of the transformed colonies in the two dishes. Comparison (ii) is for all the reseeded groups. The count for any contaminated dish in group III was assumed to be the same as its uncontaminated companion dish. No correction was made in group IV for the fact that only 80% of each culture was reseeded.

condition. In the present experiments, 600 rad produced an initiated state that survived unchanged for at least 13 cell generations, but others have shown that the initiated state produced by exposure to $1.0 \mu\text{g ml}^{-1}$ methylcholanthrene is unstable, with about a quarter of the cells reverting to normality in each generation³. Similarly, in experiments reported elsewhere⁹⁻¹¹, we have found that treating initiated cultures with phorbol esters will greatly increase the number of transformants if the initiation was with 100-400 rad, but has hardly any effect after 600 rad; this too suggests that there are degrees of initiation. At present, the most likely hypothesis is that the initial step consists of some change in the pattern of gene expression that, like the SOS response of bacteria treated with mutagens, determines the frequency of subsequent rare genetic events. Even though initiation is much more stable than the SOS response, this hypothesis is supported by our finding that certain protease inhibitors, which are known to block the SOS response¹², will reverse the initial step in transformation¹³.

In contrast, the second step is a rare, random event, occurring in slightly less than one in a million descendants of cells that have taken the first step. In the experiment reported here, the largest clone of transformed cells did not arise until 6 cell divisions after X-irradiation (that is, about 7 generations before the culture was reseeded) and most of the clones arose after 12-13 divisions. Thus, the second step cannot be the direct result of the lesions produced in DNA by the initial dose of radiation. We have observed (unpublished experiments) that the number of transformed foci arising in cultures derived from cells fully initiated with 600 rad of X rays cannot be significantly raised by treating the cultures as they approach confluence with mutagens such as X rays or UV light. If the second step is a mutation (as its frequency and random distribution might sug-

gest), it must represent an unusual class of genetic event that is not readily driven by mutagens.

Although the nature of the steps in the neoplastic transformation of established, immortal cell lines by carcinogens is not understood, what appears to be kinetically a rather similar sequence of events has been observed for radiation-induced recombination in yeast¹⁴. Additional steps would probably be needed to transform normal cells *in vivo* and the kinetics of these steps have not yet been studied in similar detail.

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1. Mondal, S. & Heidelberger, C. *Proc. natn. Acad. Sci. U.S.A.* **65**, 219-225 (1970).
2. Kennedy, A. R., Fox, M., Murphy, G. & Little, J. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7262-7266 (1980).
3. Fernandez, A., Mondal, S. & Heidelberger, C. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7272-7276 (1980).
4. Backer, J. M., Boerzig, M. & Weinstein, I. B. *Nature* **299**, 458-460 (1982).
5. Hall, E. J., Rossi, H. H., Zaider, M., Miller, R. C. & Borek, C. in *Neutron Carcinogenesis* (eds Broerse, J. J. & Gerber, G. B.) 381-405 (Commission of the European Communities, Luxembourg, 1982).
6. Barrett, J. C. & Elmore, E. in *Handbook of Experimental Pharmacology* (eds Andrews, L. S., Lorentzen, R. J. & Flamm, W. G.) (Springer, Berlin, in the press).
7. Luria, S. E. & Delbrück, M. *Genetics* **28**, 491-511 (1943).
8. Lea, D. E. & Coulson, C. A. *J. Genet.* **49**, 264-285 (1949).
9. Kennedy, A. R. & Little, J. B. *Carcinogenesis* **1**, 1039-1047 (1980).
10. Kennedy, A. R., Mondal, S., Heidelberger, C. & Little, J. B. *Cancer Res.* **38**, 439-443 (1978).
11. Yavelow, J., Finlay, T. H., Kennedy, A. R. & Troll, W. *Cancer Res.* **43**, 2545-2549 (1983).
12. Meyn, M. S., Rossman, T. & Troll, W. *Proc. natn. Acad. Sci. U.S.A.* **44**, 1152-1156 (1977).
13. Kennedy, A. R. & Little, J. B. *Nature* **276**, 825-826 (1978).
14. Fabre, F. & Roman, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1667-1671 (1977).
15. Kennedy, A. R. & Little, J. B. *Cancer Res.* **41**, 2103-2108 (1981).

What we know about acid rain

Kenneth Mellanby

Acid Rain: A Review of the Phenomenon in the EEC & Europe.

Compiled by Environmental Resources Limited.

Graham & Trotman, Sterling House, 66 Wilton Rd, London SW1/Unipub, POB 433, Murray Hill Station, NY 10157: 1983. Pp.159. £12.50, \$23.

IN 1982 Environmental Resources Limited (ERL), a private consultancy in London, received a contract from the Commission of the European Communities (EEC) to survey the problem of "acid rain". ERL rightly interpreted their brief to include all acid pollution produced by industry and the burning of fossil fuels. They also considered ozone, another pollutant of industrial origin, which may have its own harmful effects but which may also play a part in the generation of strong acids from other chemicals in the atmosphere.

The result, now published in book format, is a succinct and practical account, summarizing a vast amount of information in 160 pages, which include 15 of references. This bibliography is selective, but contains most useful publications up to 1982. With this volume available, there is no longer any excuse for journalists to continue to produce the uninformed nonsense they have inflicted on the public in recent months.

It is clear that when we are considering the direct phytotoxic effects of acid emissions, it is when they are in the form of gases, and to a lesser extent, of particles, that they are important. It is dry deposition that does the damage. This is something with which we have been familiar for more than a hundred years, but recently opinions may have become confused by allegations that polluted rainwater — "acid rain" — may be even more important. ERL give all the facts to allow the reader to disentangle the effects of dry and wet deposition. They make clear that, as far as direct damage to foliage and buildings is concerned, the acid rain commonly falling in the south of England has little harmful effect. Its acidity is thus something of a red herring, even though it is the property which has caused the greatest public disquiet.

Acute phytotoxicity in Britain has in fact greatly decreased in recent years, though this point is hardly made in the report. The improvement is generally attributed to the Clean Air Act of 1956, but was mainly caused by the substitution of clean, convenient natural gas for dirty coal in homes and industry. It has also been achieved by blowing the flue gases from coal-fired generating stations (still the biggest sources of sulphur dioxide) up tall chimneys so that they are mixed with air and thus diluted below the levels where plants are severely harmed.

This policy, obviously beneficial to Britain, has been severely criticized elsewhere. For instance a recent Swedish

leaflet, handed out to tourists visiting that country, alleged that Britain is being selfish, reducing pollution at home by increasing it abroad. Fortunately this is not strictly true. Even with low chimneys only a small (though very damaging) amount of sulphur dioxide is deposited locally; most of it mixes into the atmosphere. So though higher chimneys produce important local reductions of pollution, they do not put much more sulphuric acid into the atmosphere; thus the gaseous concentrations a hundred or more miles from source are much the same whatever the height at which the emission originally took place.

The Swedish countryside's secret: The acid rain is slowly killing it.

Sulphur and nitrogen from the combustion of oil and coal fall with the rain, in the form of acid. This acid damages both the land, and the water. 18,000 lakes in Sweden have already been damaged by acid rain. Thousands of them are practically dead. If nothing is done, many more will soon be badly affected. The soil, the forests, and the subsoil water are also being damaged.

Acidification is our worst single environment problem. And yet many people are still unaware of this. It cannot be seen in the countryside. An acidified lake looks clean and clear, even if it has been poisoned to death.

Acidification is an international environment problem. That is why we say that what is happening in the Swedish countryside matters to you. Air pollutants are carried across countries, and across national frontiers, by the winds. Three quarters of the sulphur falling over Sweden originates from other countries.

The only way of saving our countryside from acidification is to reduce radically discharges in Europe, primarily of sulphur. This can only be achieved by international collaboration. And that is why we are concerned to tell you about acidification — by making the problem known in your own country, you will be helping us to solve it!

Acid rain and international relations — detail from a leaflet, intended for tourists, issued by Swedish conservation and angling bodies.

Until recently, it was thought that the policy of "dilute and disperse" had been completely successful, both locally and on a global scale. However, we now find that some damage may occur to crops and trees from levels of gases below those where acute damage is evident. This seems particularly true when the oxides of sulphur and nitrogen mix with ozone. Nevertheless this is still a local problem, occurring within a radius of perhaps up to two hundred miles from the pollution source. Further off, with greater dilution, no further direct damage to plants or to buildings occurs. Until recently we thought that these very dilute gases were completely harmless, and

that the pollution problem had largely been solved.

Unfortunately, it has not proved to be so simple. In Scandinavia, for instance, the levels of sulphur dioxide, a substantial part of which comes from Britain, are indeed low, and cause no direct damage to plant life. In fact very delicate foliose lichens, well known biological indicators of air pollution, flourish. But lakes and rivers are becoming acidic, fish are disappearing, and in some parts of Europe (though not in Scandinavia) trees appear to be damaged. This is all blamed on acid rain, and as the chemicals producing the acidity blow from one country to another, the problem is an international one.

A major cause of damage is believed to be that as the air passes from one area to another, perhaps taking several days to do so, the sulphur dioxide is transformed slowly to sulphate and sulphuric acid. This is washed out of the air, and may then acidify lakes, streams and soils where calcium levels are low and there is poor buffering. The rain itself, even when it contains sulphuric acid, is so dilute that it has no direct phytotoxicity; it is the indirect effect on the soil which is thought to be important.

But there are still many uncertainties. We simply do not understand the quantitative relationships between emissions and their environmental effects. One American report showed a linear relationship, with damage closely correlated with the amounts of pollution emitted. European studies have shown no such correlation. This disparity may be because different phenomena are being investigated. The local effects within the UK of air pollution generated in Britain are roughly proportional to emissions, certainly within a radius of a couple of hundred miles from source. But more distant effects, involving chemical transformations whose rate depends on several factors, cannot be determined so easily.

The value of this publication is that it summarizes the existing information, and shows how little we know and where research is needed. I cannot describe the situation better than by quoting from the summary:

However, it has not been unequivocally established that these environmental impacts are caused by acid pollutant emissions, nor is the relative importance of other factors properly identified. Also considerable further investigations are required to understand the mechanism involved. Nevertheless circumstantial evidence would suggest that acid emissions and their subsequent chemical transformation and precipitation are at least a partial contributory cause to the observed effects and may be giving rise to as yet unidentified impacts, some of which could be irreversible. □

Kenneth Mellanby is Chairman of the Working Group on Acid Rain of the Watt Committee on Energy. This committee represents 60 British professional engineering and scientific organizations.

Ageing friends at a congenial meeting

Alex Comfort

Aging: An Exploration. By David Barash.
University of Washington Press: 1983.
Pp.240. \$14.95, £12.75.

POPULAR books on ageing are rather like conferences on a recondite area of research — they tend to be parades of familiar faces. One greets the quotations and examples like old friends and looks out for those who should be there. Professor Barash's book is an entertaining reworking of Visscher, Ernest and other — largely prescientific — writers on age. Unfortunately, while the literary background is colourful and some of the social comment is acute, the corpus of modern gerontological research has largely passed Barash by: he is still going on about Orgel's error hypothesis and Bidder on growth, and about the Vilcabambans and Abkhasians, when the focus of

potential application has shifted to the hypothalamus, to immunology and to other areas largely ignored here.

The book is well-written, amusing and accurate in terms of the gerontology of, say, 1960 (though Barash regrettably attributes Frederick the Great's classic exhortation in battle — "Sons of bitches! D'you want to live forever?" to an American sergeant). But it fails altogether to project ageing research as the going concern which it is: there is little or nothing about research prospects, and a good deal too much about the history of quackery. This is partly because the earlier gerontologists made their point and entered the nature of ageing in the agenda of their colleagues — in neurotransmitter studies, for example — but it makes the book a little dated, and unlikely to inspire younger researchers to take up the subject and secure clinically applicable results. □

Alex Comfort is Consultant in Geriatric Psychiatry to the Veterans' Administration, Los Angeles, and an Adjunct Professor at the University of California, Los Angeles.

Dynamics of dizzy nuclei

Neil Rowley

Fast Nuclear Rotation.
By Zdzislaw Szymański.
Oxford University Press: 1983. Pp.250.
£35, \$39.

IN ATOMIC and solid-state physics much can be learned about the internal dynamics of a system by the application of external electric and magnetic fields. The nucleus, however, is a strongly bound object and, in the laboratory, it is not possible to create fields that are sufficiently strong to perturb its internal structure. One extremely fruitful alternative is to set the nucleus spinning and see how its structure responds to the resulting inertial forces. Our knowledge of nuclear structure has greatly increased over the past ten years or more through the application of this idea, and in *Fast Nuclear Rotation* Szymański has set out to document the relevant theoretical and experimental advances.

For a rotating system one can generalize the concept of a quantum mechanical ground state by talking of the configuration which has the lowest energy for a given value of the angular momentum. Equivalently the set of states defined in this way may be thought of as having the largest possible spins for given excitation energies, and are referred to as the "yrast" states of the system. ("Yrast", as Szymański explains, is the superlative of the Swedish "yr", meaning dizzy.)

For sufficiently rapid rotations the entire nuclear shape will be modified and may

undergo violent convulsions in an attempt to minimize its energy while accommodating large amounts of angular momentum. It is finally unable to find any equilibrium configuration and will be torn apart by the enormous centrifugal forces present. The book takes us up the entire yrast line from the low-spin region to this ultimate demise into fission fragments. Along this path many fascinating changes in internal structure take place, and it is the observation of these nuclear "glitches" (e.g. sudden changes in the moment of inertia) and their interpretation in terms of the underlying nucleon motions which is the aim of the field of nuclear high-spin states.

As is described in the book, the most usual way of producing a rapidly rotating nucleus is by the so-called fusion-evaporation mechanism in which two nuclei are caused to collide at energies high enough to overcome their mutual Coulomb repulsion. The fused system produced in this way has a high angular momentum and a large excitation energy. Indeed for any given spin, the energy of the compound nucleus will be significantly higher than the corresponding yrast energy and the system may be thought of as being hot. Just like a hot classical liquid, the droplet of hot nuclear matter cools by "evaporation", i.e. by rapid emission of neutrons, protons and alpha particles which carry away large amounts of energy but little angular momentum. (In addition to producing high-spin states the evaporation process may also be used to produce exotic β -unstable isotopes.) On reaching a lower temperature the nucleus can no longer emit particles and the subsequent energy and spin losses must take place via series of γ -decays into and through the yrast states or

down the many other "side-bands", i.e. sequences of higher energy rotational states. It is the experimental observation of the ensuing γ -cascades which is the key to the elucidation of nuclear structure at high spins. The sophistication of experimental devices and techniques has grown enormously in recent years, allowing one to resolve individual γ -decays from states with spins in excess of forty units of angular momentum. Indeed many significant advances have been made since the manuscript of *Fast Nuclear Rotation* was completed, though this is a reflection of the vitality of the subject rather than too serious a criticism of Szymański's treatment in the book.

Many of the phenomena associated with nuclear rotations can be thought of in a semiclassical way. Indeed the very phrase "nuclear rotations" implies that it is possible to think of the nuclei in question as deformed objects rotating with some classical angular velocity, which is in turn related to the frequency of the emitted γ -rays. Although typical rotations may seem rather fast ($\sim 10^{20}$ revolutions per second) they correspond to velocities of the nuclear surface significantly less than those of the individual nucleons through the nuclear interior. It is, therefore, meaningful to think of a particle moving in an average field generated by all the others, and of this field rotating in space. This notion leads to the cranked shell model for high-spin states and Szymański discusses many successes of this model in detail. For example the phenomenon of backbending (a decrease in rotational velocity accompanying an increase in angular momentum) is explained in terms of the effect of the Coriolis force on the individual nucleons. In the nuclear ground state nucleons tend to be coupled in pairs, with zero total angular momentum, while the Coriolis force tends to align their individual angular momenta with the axis of rotation. This increases the total spin of the system even though its collective rotational velocity may drop as a given pair is broken up. The interplay between such collective and single-particle effects is central to this area of study and is well brought out in the book.

In order to understand the relevance of high-spin phenomena it is of course essential to have a certain knowledge of more conventional nuclear-structure physics. Szymański admits that some of the important theoretical ingredients have been treated rather briefly in his Chapter 1. Although this is mitigated to some extent by copious references, the uninitiated will find the book tough going. For those with a reasonable working knowledge of nuclear structure, however, it will be essential reading. □

Neil Rowley is in the Theory and Computational Science Division at the Science and Engineering Research Council's Daresbury Laboratory, Warrington.

Photoreceptors for the beginner

Aubrey Knowles

Photoreceptors: Their Role in Vision.

By Alan Fein and Ete Z. Szuts.

Cambridge University Press: 1983.

Pp.212. Hbk £17.50, \$34.50;

pbk £7.95, \$13.95.

OUR knowledge of retinal photoreceptors has increased considerably over the past 20 years, and our present ideas about their function have come from a variety of disciplines ranging from anatomy through chemistry to physics. This book meets a need by providing a comprehensive review at a fairly elementary level. The fact that it appears in the IUPAB Biophysics Series should not put off intending readers: the volume could equally well have been included in a series on zoology or chemistry.

Vision is a sense common to most animals, and it is now clear that the photoreceptors of all species have structural similarities and their visual pigment molecules are closely related. However a gulf exists between workers on the vision of vertebrates and those dealing with invertebrates, due to the different visual characteristics observed and to the use of different techniques. The authors have made a good job of pulling together a wealth of information from these two fields, but this has of necessity involved the critical selection of material.

My principal criticism of the book is of bias in this selection that arises from Drs Fein and Szuts's interest in invertebrate

vision. While this may merely reflect my interest in vertebrate photoreceptors, most students take an anthropomorphic view of vision, and can best tackle the subject as an extension of their own experience. The authors recognize this in the introductory chapters, where human visual performance is stressed, but this approach is abandoned as the text develops. In Chapter 8, which covers adaptation, vertebrates are barely mentioned. Cone receptors, colour vision and the visual performance of vertebrates receives poor coverage, although this area is of general interest and is the subject of active current research. The book thus attempts a useful service in bringing together the very different visual characteristics of insects and man, but fails to sustain this approach through to the end.

In another way the balance of the book is also not quite right, the early chapters being cluttered with detail while the later chapters become shorter and more cursory. Much of the early detail is irrelevant and

some is contentious — for example, the conjectured sensitizing pigment in fly receptors on p.78 — while recent important work on single cell electrophysiology and the molecular mechanisms of transduction and adaptation are all compressed into the final 30 pages. The references are sensibly arranged at the end of the book and form a useful reading list. Unfortunately this list omits some useful recent reviews and contains a number of outdated and trivial references which will not be helpful to a newcomer to the field.

In summary, the book is a useful addition to the literature of vision, and will be helpful to students and research workers entering the field; I will recommend it to our second-year students in future. It is nicely produced though some of the figures are poor. □

Aubrey Knowles is a Research Fellow in the Department of Biochemistry, University of Bristol.

The microfilament system

Uno Lindberg

Mechanisms of Cell Motility:

Molecular Aspects of Contractility.

By Peter Sheterline.

Academic: 1983. Pp.180. £14, \$23.

RESEARCH on the structural organization of eukaryotic cells in general and cell motility in particular was revolutionized by the development of antibodies against actin by Lazarides and Weber in 1974. Discovery of these antibodies led to a startling new picture of the distribution of actin in different kinds of cells and made it clear that chemo-mechanical transduction by the actomyosin system might be a fundamental process common to all eukaryotic cells. Exciting findings followed rapidly, and within a few years it became evident that eukaryotic cells have a complex and dynamically changing cytoskeleton consisting of finely tuned filament systems (microfilaments, microtubules, and intermediate filaments) through which the life processes can be directed and performed in a precise way. Peter Sheterline's book deals primarily with the microfilament system.

The molecular mechanisms underlying cell motility are still largely unknown. Many proteins which interact with the microfilament system have now been isolated, but the observations concerning their activity *in vitro* give only vague answers to several of the outstanding problems — how microfilaments are formed; how filopodia, microvilli and stress fibres are built by packing of actin filaments in paracrystalline bundles; how membrane lamellae extend themselves

from the surface of cells and might cause the observed translocations; and how all of these phenomena are regulated.

When lecturing on these matters I usually begin by describing the diversity of motile activity expressed by eukaryotic cells but emphasize that there might be a common basic mechanism. But to consider this it is necessary to have a relatively detailed picture of the organization of the individual structural elements of different types of cells and the different filament systems. After covering that I explain the difficulties involved in correlating the molecular organization of the cell structure with the behaviour of cells. Only then do I tackle the biochemistry of the components of the filament systems. I feel it is difficult for the beginner to see the molecular details but not lose track of the overall structural organization and behaviour of the cell.

Peter Sheterline has organized his book the other way around. He starts with a thoughtful discussion of the proteins involved in the microfilament system. Then he deals with the ultrastructural organization and with possible mechanisms. One could approach the book by taking a good look at the pictures of cells provided in Chapter 6, and then read Chapters 1–5 to find out more about the proteins involved. Then one might study carefully the concept of "contractile networks", but without taking the different models presented too seriously — they may be far from the reality of the living cell. Unfortunately, the author makes no attempt to tell how the story has developed, and he makes few references to original observations. So while there is something in the book to exercise the specialist, the newcomer should look elsewhere for an introduction to this field. □

Uno Lindberg is a Professor at the Wenner-Gren Institute, University of Stockholm.



Adaptive radiation as displayed by Hawaiian sicklebills. The illustration is taken from *Darwin's Finches* by David Lack, recently re-issued by Cambridge University Press with notes by Laurene Ratcliffe and Peter Boag. Price is hbk £19.50, \$39.50; pbk £7.95, \$13.95.

Talk of science and philosophy

Donald MacKay

The Philosophy of Popper.

By T.E. Burke.

Manchester University Press: 1983.

Pp.220. £6.50, \$8.

IN a year that has seen the publication (at last) of Sir Karl Popper's famous three-volume *Postscript* (see *Nature* 300, 663-664, 1982; 302, 357-358, 1983), there would be little need for yet another summary of his philosophical position. What Dr Burke has provided (despite his textbookish title) is something quite different. Recognizing that "his own works are by far the best sources of information about the context of [Popper's] thought" (p.vii) he has chosen instead to take some of Popper's key ideas as a starting point for critical enquiries which explore much wider territory. In the process he brings out both the strengths and the weaknesses he sees in Popper's massive contribution to contemporary thinking about the nature of science and scientific reasoning, of human society and human freedom, and indeed of the philosophical enterprise itself.

The book falls into five sections, concerned respectively with the philosophy of philosophy, the philosophy of science, relativism and truth, historicism, and freedom and values. In each, Popper's characteristic emphases are noted, with the help of representative quotations; but on the whole the reader is expected to be familiar with their original context, and to be interested mainly in the way they relate to other strands of contemporary thought.

Popper sees philosophy as a critical or mind-clearing response to crises that arise (typically) in other fields of interest. As such it has genuine problems to tackle; and Popper had no time for the Wittgensteinian idea that its goal should be to dissolve rather than solve them. But what Burke asks is whether, after all, Popper and Wittgenstein had as much to quarrel over as they thought; and he argues that Popper's own philosophizing "can equally well be presented as almost classical examples of Wittgensteinian philosophy". Popper's famous refutation of historicism, for example, "hinges on the claim that, given a sufficiently careful description of the relevant disciplines, and drawing of the demarcation-lines between them, the temptations of historicist programmes disappear" (p.25).

For Popper our main concern, whether as scientists or philosophers, is, or ought to be, the search for truth; no mere instrumentalism could do justice to the drive behind the scientific enterprise. Yet paradoxically he is also famous for the doctrine that though science is seeking truth, it can never have logically sufficient

grounds for claiming to have found it. Induction, in particular, he has dismissed as "a myth". Burke, while sympathetic to Popper's reasons for such radical scepticism, argues that "his taste for the dramatic has led him to overstate his case" (p.48). Burke insists that for any serious attack on falsehood or ignorance, we need a basis of established truths, however limited, which can and must be secured in ways that avoid the corrosion of universal scepticism. Without this, he argues, Popper's uncompromising rejection of relativism runs into logical difficulties.

What Popper rejected, of course, was not the principle of relativity as such, but the tendency, especially among social scientists, to turn it into a denial of objectivity. "By relativism", he said, "I mean the theory that the choice between competing theories is arbitrary" (cited on p.82).

Popper's terminology does not make it as clear as he might that a thorough-going relativist can also be a thorough-going objectivist, provided that there are objective transformations linking statements that are valid from different standpoints. Burke, however, goes further and maintains that "there is a case for rela-

tivism in the form in which Popper denounces it" (p.86); but the case he makes is not particularly strong.

Scientific theories can indeed be surprisingly resilient in face of contradictory evidence; but this does nothing to justify Feyerabend's claim (cited on p.70) that "we can turn science from a stern and demanding mistress into an attractive and yielding courtesan who tries to anticipate every wish of her lover". It does, however, as Burke points out, tend to soften the sharp epistemic contrast usually drawn by Popperian fallibilists between scientific and metaphysical doctrines.

These examples typify Burke's treatment of his theme — respectful and sympathetic, but refreshingly independent, and eager to report what he can see when standing on the shoulders of his chosen giant. He handles language with a craftsman's skill and precision, and though seldom humorous is never dull. Reading him will be a stimulating experience for anyone who, having absorbed Popper's eloquent message, wonders what remains to be said. □

Donald MacKay is Emeritus Professor of Communication and Neuroscience at the University of Keele.

Planning ahead

Warren R. Jones

Birth Control Technologies: Prospects by the Year 2000.

By Michael J.K. Harper.

Heinemann Medical/University of Texas Press: 1983. Pp.270. £15, \$27.50.

THIS timely and comprehensive volume highlights the salient question surrounding global activities in fertility regulation. The social and personal imperatives for effective family planning are as pressing now as they have ever been, but the strategic priorities remain unclear — whether to concentrate finite resources on improving the distribution and acceptability of existing contraceptive methods, or to promote further research into new technologies.

Contraception research and development has now reached a watershed. In recent years, partly as a result of the economic recession, there has been a relative decline in the funding of goal-orientated research into reproduction. This decline began at a time when at least some new contraceptive techniques had reached a critical stage of development. Further progress will now only be made either by dint of serendipitous breakthrough or by more investment of high-risk capital by international agencies and pharmaceutical companies.

The book is built around Dr Harper's critical perception of the current state of the contraceptive art and of prospects for the practical application of new and

improved birth control technologies by the year 2000. Each method — current, new and potential — is comprehensively reviewed and supported by an up-to-date and extensive, though critical, survey of relevant literature. The author's predictions are grouped in temporal categories of implementation: highly likely before 1990; possible, but prospects doubtful by 1990; unlikely before 1990 but possible by 2000. Most promise is seen in the area of female contraception with little to offer for the male. The most likely new technology strategies to reach practical application in the next decade are steroid implants, new injectables, better IUCDs, vaginal rings, prostaglandins for abortion and non-steroidal anti-ovulation agents. The fate of radically superior new methods such as contraceptive vaccines, menses-inducing pills or tampons, and pills or injections for the male is uncertain at present.

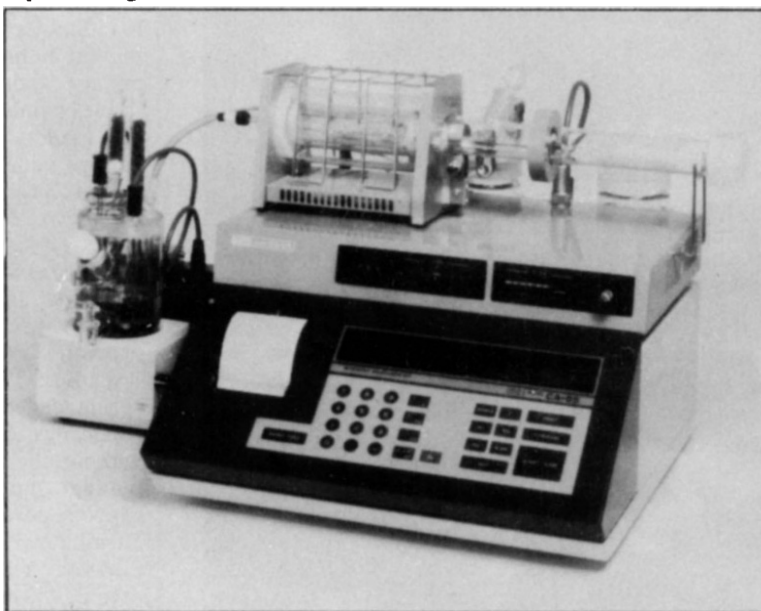
All health professionals who are concerned with the control of human reproduction would do well to read *Birth Control Technologies*. It is scientifically impeccable and, at the same time, eminently readable. In some senses, however, it is depressing. Our contraceptive armoury has progressed little beyond modification of steroidal methods, medicated IUCDs and mechanically simplified contraception. The promise of the future is uncertain, but insofar as advances in science are predictable Dr Harper has given us a blueprint for the next two decades and possibly beyond. □

Warren R. Jones is Professor in the Department of Obstetrics and Gynaecology at Flinders University of South Australia.

The products featured in this week's selection include an air purifier, a refractive index detector and a digital colorimeter

● A new range of all-diffused, fast recovery diodes has been introduced by Westcode Semiconductors. The CXC 924 series is based on 50 mm diameter silicon. It has been developed for use in inverter and chopper circuits. This new diode series is characterized by a repetitive voltage rating of between 1,600 and 2,500 V and an average forward current of 1,265 A at a heat sink temperature of 55°C. Maximum forward current is 2,450 A.
Circle No. 100 on Reader Service Card.

● The Mitsubishi CA-05 moisture meter offers sensitivity to 0.1 μg H_2O . It measures moisture by applying coulometry to the Karl Fischer titration method. The micro-processor controls eight calculation formulae. Samples containing interfering substances and solids insoluble in Karl Fischer reagents are measured by attaching the VA-05 water vaporizer to the CA-05 moisture meter. The VA-05 removes the water from the sample, then transports it to the CA-05 for Karl Fischer titration. The CA/VA combination has a maximum titration speed of 2 mg H_2O min^{-1} and a range of 10 μg to 100 mg H_2O . Precision is $\pm 5 \mu\text{g}$ from 10 μg to 1 mg, and 0.5% over 1 mg. A 16-digit alphanumeric display presents calculation results in p.p.m. and percentage H_2O , as well as logging the year, month, day, hour and minute. An integral 20-digit thermal dot matrix printer provides a complete record of calculations.
Circle No. 101 on Reader Service Card.



The CA-05 moisture meter from Mitsubishi can detect 0.1 μg of water.

● A refractive index detector, the SP 6040, is now available from Spectra-Physics. The SP 6040 is a deflection-type differential refractive index detector. Its long focal length increases sensitivity. A temperature control system is built in.
Circle No. 102 on Reader Service Card.

● The 125-test bacteriuria screening kit from Turner Designs is now available from Techmation. The assay is based on the micro-ATP measurement of viable organisms and uses only 50 μl of luciferin-luciferase. The system is claimed to have a false negative rate of around 2% and a false positive rate of 20%. No centrifugation or somatic ATP extraction is required. The model 20 can be used with both the new Turner Designs kit and with most *in-vitro* diagnostic kits.
Circle No. 103 on Reader Service Card.

● The Rodwell Ensign is a free-standing, vertical-loading autoclave with a chamber space of 100 litres. The system does not have to be 'plumbed in', which means that installation only requires an electrical supply. Water that has condensed from the chamber is collected through an integral catchment system. This minimizes water consumption and removes the need for drains and collection vessels.
Circle No. 104 on Reader Service Card.

● Romicon has developed a system for the treatment of water and other fluids before reverse osmosis. The Romicon RO treatment system has wide channel tubes, hollow fibre membranes and operates in a shell and tube geometry at low pressures. It has membrane filters of approximately 0.005 μm .
Circle No. 105 on Reader Service Card.

Circle No. 105 on Reader Service Card.

● Micro Instruments has produced a glass microelectrode puller. This new vertical puller is an automatic double-pull type which will take 1 to 4 mm diameter glass tubes. The setting controls allow the heat and the magnets for the initial and the second pull to be adjusted, depending on the thickness of glass tubing being used. Further adjustments are provided to control the heating off time and the pulling time. The glass tubing is held in chucks that are controlled by a 'one-touch' clamping device. The instrument is supplied with a range of chucks and heater coils.
Circle No. 107 on Reader Service Card.

● Astell Hearson has increased its Swiftlock range of autoclaves with the new Swiftlock series 3000. These autoclaves are designed to enable processing of both media and discard material. Three independent adjustable programs are selectable from the front panel and all adjustable controls including a fault reset button are behind a lockable, perspex panel. Supercool models are also being produced.
Circle No. 108 on Reader Service Card.

● Jenway has developed a new digital colorimeter. The Jenway PC01 colorimeter operates in three measurement modes: transmittance, from 0 to 100%, to a resolution of 0.1%; absorbance, which is electronically linearized, from 0 to 1.999 to a resolution of 0.001; and two concentration ranges which provide coarse and fine measurements over the absorbance readings. In this mode the instrument, using most methodologies, provides a direct reading of solution concentration. The PC01 is supplied with eight integral filters to cover the wavelength range 400 to 710 nm. The instrument's bandpass is typically 40 nm.
Circle No. 109 on Reader Service Card.

Circle No. 109 on Reader Service Card.

● A catalogue of specialized equipment for rodent research is now available from Harvard Bioscience. Areas covered in the catalogue include respiration, animal surgery, and housing.
Circle No. 110 on Reader Service Card.

Circle No. 110 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● A new range of Mystaire ward and laboratory air purifiers is now available from Heat Systems Ultrasonics. Interchangeable packs of chemisorbents, activated charcoal and impregnated charcoal are used in conjunction with standard mechanical filter packs to remove specific airborne contaminants.

Circle No. 111 on Reader Service Card.

● Racal Safety has added a power-assisted respiratory system to its Dustmaster series. The Dustmaster 7 includes a mask weighing 50 g with all-round vision. The mask receives a clean air supply through a hose attached to a belt-mounted motor/fan filter.

Circle No. 112 on Reader Service Card.

● Horwell's new Accusplit 920 XP digital LCD quartz wrist watch offers several scientific functions. The countdown timer, pre-settable up to 15 h 59 min, sounds an alarm for 5 s on expiry of the countdown period. Its time-of-day alarm sounds when the pre-set alarm time is reached, but a day exclusion facility stops the alarm going off on days when it is not required. The stop-watch facility times up to 12 h, unless previously stopped, in minutes, seconds and one hundredths of a second up to 20 min and thereafter in hours, minutes and seconds. The watch also tells time of day to the nearest second along with month, date and day of the week. There is an optional hourly bleep.

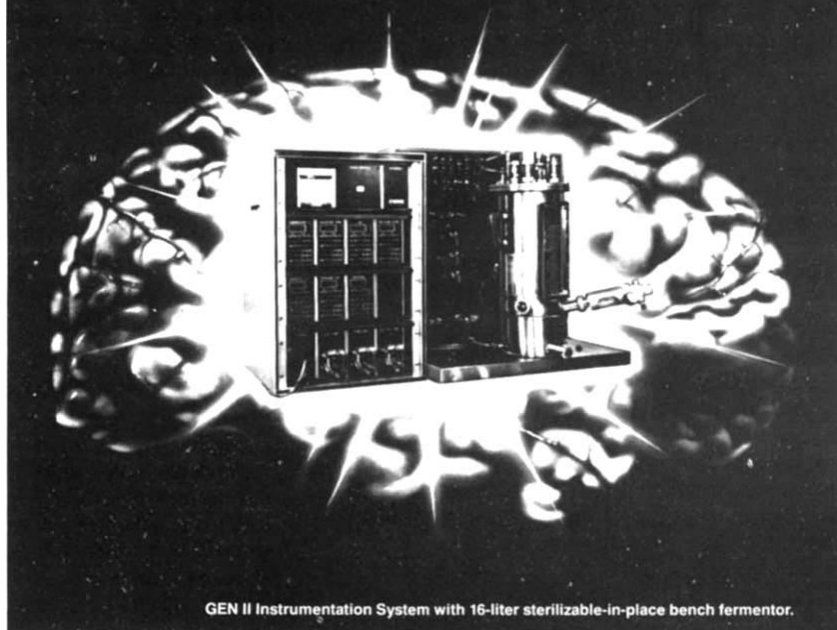
Circle No. 113 on Reader Service Card.

● Matheson has produced a new electro-pneumatic system that will automatically purge hazardous gas systems. The system is microprocessor-controlled so that valve sequence, number of purge cycles and cycle time can be programmed. The system also responds to pressure, confirmation of valve operation and completion of previous operations. The controller features a shutdown mode in which all valves close when there is a power failure or electrical component malfunction. The purging hardware portion of the system can be up to 200 feet from the controller. The system uses pneumatically-activated Matheson Packless valves which will operate against a cylinder pressure of 2,500 p.s.i. in either direction.

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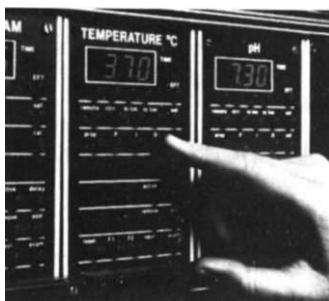
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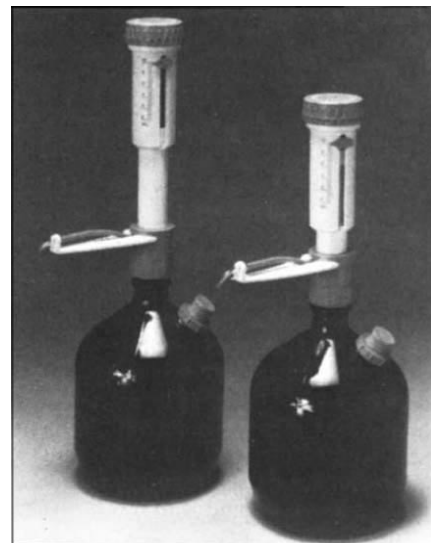
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Key skills and employment growth

Richard Pearson*

For countries left behind by the rapid progress in electronics, prospects for employment will be poor.

THE electronics market is very competitive and is being increasingly dominated by US and Japanese producers, with the Europeans coming a poor third. For example, more than half the world's semiconductor market is supplied by ten companies, five American, four Japanese and one European.

The race is now on for the "fifth-generation computer", and with increasing technical and product integration, "chips" and their derivatives are playing an increasingly critical role in the development of the electronics, manufacturing and service industries. While the semiconductor industry is important in its own right, with world sales of well over \$15,000 million a year and a workforce of about 250,000 (Fig.1), the dependent electronics-related markets are of course far bigger, employing several million people.

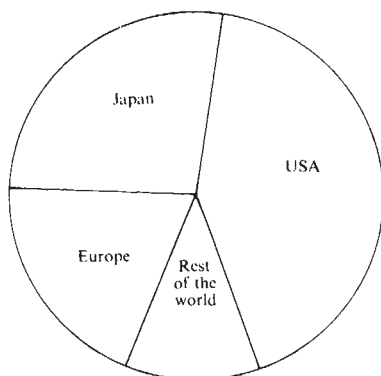


Fig. 1 Manpower in the semiconductor industry. (Institute of Manpower Studies estimates.)

Although the semiconductor industry is particularly sensitive to the economic cycle, it is now experiencing a sharp upturn, and all the forecasters agree that demand will grow significantly in the long term, with output possibly doubling by the end of the decade. Success in these micro-electronics markets means large numbers of jobs in the future, yet their success will be critically determined by the availability of a few thousand people with key skills today.

In California, in "Silicon Valley", the birthplace and home of the industry, the companies see two chief threats, growing Japanese competition and shortages of skilled manpower. The recent US Office of Technology Assessment (OTA) report (*Nature* 306, 307; 1983) may have helped to

put the general Japanese threat into perspective but the problems of recruitment are ever present and expected to get worse. In "Silicon Valley", crowded conditions, soaring house prices, rising compensatory salary levels and an inability to attract sufficient skilled staff are leading the major producers to consider moving their factories elsewhere both in the United States, in areas such as Colorado and Austin, Texas, and also in Europe with its rapidly growing semiconductor market.

Semiconductor manufacturers everywhere are struggling with a shortage of key skills and the need to develop a pool of appropriately experienced staff for the future. Thus while an electronics engineer or designer may be highly productive at the relatively young age of 24 or 25, much of his or her necessary experience will have been developed in the two or three years spent in the industry after graduation. Many of the more general skills are interchangeable with the electronics industry, enabling a new or rapidly expanding company which pays top salaries to "poach" experienced staff from a wider pool. However, in the more specialized areas of circuit design, such as very-large-scale integrated circuit (VLSI) design and in process engineering (that is, managing the production line), such skills are only developed on the job, in a semiconductor company. The high costs of equipment and the need to reinvest every three to five years preclude higher education, for example, from contributing effectively to the future supply of experienced people through undergraduate and postgraduate education.

Given the small numbers with such skills, fewer than 2,000 in Europe for example (Fig.2), there are great advantages for semiconductor companies in "clustering" together, facilitating an interchange of skills. This also helps in recruiting staff who can change jobs without relocating. Much of the success of Silicon Valley is put down to the rapid interchange of staff between companies and general ease of career mobility. Such clusters occur "spontaneously", as in California, along Route 128 in Boston, and along the M4 in England. They can also be encouraged by intervention as in Scotland, which is now, through inward investment, a major world electronics centre. Indeed the Scottish Development Agency is capitalizing on this ease of job changing and career development in a small area as part of its recruit-

ment campaign to attract electronics skills to Scotland.

The electronics and related industries are seen as one of the few certain growth sectors for the future. Most governments are therefore encouraging the development of their indigenous industries and are seeking to attract "inward investment" by electronics-based companies. Advances in automation mean that much of the growth in output is being matched, and sometimes overtaken, by increasing productivity levels. The absolute number of assembly and less skilled jobs may well shrink in the future, but the demand for key skills will continue to grow. With up to 10 or more other jobs in the industry, and many more outside it, dependent on each skilled job, the availability of key skills will continue to have a disproportionate effect on attracting and expanding employment in a particular locality.

For many of these skills, semiconductor companies can look to the much wider elec-

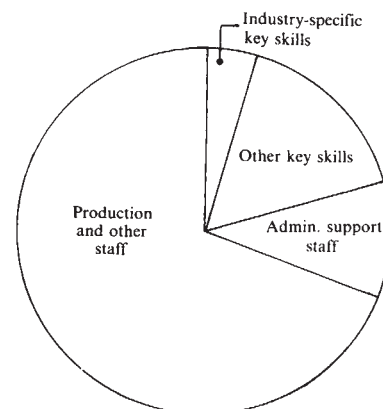


Fig. 2 Balance of skills in European industry.

tronics industry and, provided it can offer attractive salaries and conditions, employers should be able to buy their way out of problems. However, in terms of the few thousand staff needing industry-specific skills, such as VLSI design and process engineering, relevant experience, which can only be acquired in the industry, is critical. Given the long lead times involved in gaining such experience, how will the supply be increased to meet the growing needs of the future? With shortages of skilled staff already apparent, will market forces work resolve these mismatches or will manpower availability and constraints help to determine the industry's winners and losers, and hence the prosperity of particular localities over the next decade? □

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